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(54) Title: PLURIPOTENTIAL STEM CELLS

(57) Abstract: We describe a method to manipulate the phenotype of stem cells, preferably pluripotential stem cells including nucleic acids and vectors used in said methods.

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Pluripotential Stem Cells

The invention relates to a method to manipulate the phenotype of stem cells, preferably pluripotential stem cells and including nucleic acids and vectors used in said methods.

A number of techniques have been developed in recent years which purport to specifically ablate genes and/or gene products. For example, the use of anti-sense nucleic acid molecules to bind to and thereby block or inactivate target mRNA molecules is an effective means to inhibit the production of gene products. This is typically very effective in plants where anti-sense technology produces a number of striking phenotypic characteristics. However, antisense is variable leading to the need to screen many, sometimes hundreds of, transgenic organisms carrying one or more copies of an antisense transgene to ensure that the phenotype is indeed truly linked to the antisense transgene expression. Antisense techniques, not necessarily involving the production of stable transfectants, have been applied to cells in culture, with variable results.

In addition, the ability to be able to disrupt genes via homologous recombination has provided biologists with a crucial tool in defining developmental pathways in higher organisms. The use of mouse gene "knock out" strains has allowed the dissection of gene function and the probable function of human homologues to the deleted mouse genes, (Jordan and Zant, 1998).

A much more recent technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated.

Recent studies suggest that RNAi molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

The exact mechanism of RNAi action is unknown although there are theories to explain this phenomenon. For example, all organisms have evolved protective mechanisms to limit the effects of exogenous gene expression. For example, a virus often causes deleterious effects on the organism it infects. Viral gene expression and/or replication therefore needs to be repressed. In addition, the rapid development of genetic transformation and the provision of transgenic plants and animals has led to the realisation that transgenes are also recognised as foreign nucleic acid and subjected to phenomena variously called quelling (Singer and Selker, 1995), gene silencing (Matzke and Matzke, 1998) , and co-suppression (Stam et. al., 2000).

Initial studies using RNAi used the nematode *Caenorhabditis elegans*. RNAi injected into the worm resulted in the disappearance of polypeptides corresponding to the gene sequences comprising the RNAi molecule(Montgomery et. al., 1998; Fire et. al., 1998). More recently the phenomenon of RNAi inhibition has been shown in a number of eukaryotes including, by example and not by way of limitation, plants, trypanosomes (Shi et. al., 2000) *Drosophila spp.* (Kennerdell and Carthew, 2000). Recent experiments have shown that RNAi may also function in higher eukaryotes. For example, it has been shown that RNAi can ablate *c-mos* in a mouse ooctye and also E-cadherin in a mouse preimplanation embryo (Wianny and Zernicka-Goetz, 2000).

During mammalian development those cells that form part of the embryo up until the formation of the blastocyst are said to be totipotent (e.g. each cell has the developmental potential to form a complete embryo and all the cells required to support the growth and development of said embryo). During the formation of the blastocyst, the cells that comprise the inner cell mass are said to be pluripotential (e.g. each cell has the developmental potential to form a variety of tissues).

Embryonic stem cells (ES cells, those with pluripotentiality) may be principally derived from two embryonic sources. Cells isolated from the inner cell mass are termed embryonic stem (ES) cells. In the laboratory mouse, similar cells can be derived from the culture of primordial germ cells isolated from the mesenteries or genital ridges of days 8.5-12.5 *post coitum* embryos. These would ultimately differentiate into germ cells and are referred to as embryonic germ cells (EG cells). Each of these types of pluripotential cell has a similar developmental potential with respect to differentiation into alternate cell types, but possible differences in behaviour (eg with respect to imprinting) have led to these cells to be distinguished from one another .

Typically ES/EG cell cultures have well defined characteristics. These include, but are not limited to;

i) maintenance in culture for at least 20 passages when maintained on fibroblast feeder layers;
ii) produce clusters of cells in culture referred to as embryoid bodies;
iii) ability to differentiate into multiple cell types in monolayer culture;
iv) can form embryo chimeras when mixed with an embryo host;
v) express ES/EG cell specific markers.

Until very recently, *in vitro* culture of human ES/EG cells was not possible. The first indication that conditions may be determined which could allow the establishment of human ES/EG cells in culture is described in WO96/22362. The application

describes cell lines and growth conditions which allow the continuous proliferation of primate ES cells which exhibit a range of characteristics or markers which are associated with stem cells having pluripotent characteristics.

More recently Thomson *et al* (1998) have published conditions in which human ES cells can be established in culture. The above characteristics shown by primate ES cells are also shown by the human ES cell lines. In addition the human cell lines show high levels of telomerase activity, a characteristic of cells which have the ability to divide continuously in culture in an undifferentiated state. Another group (Reubinoff et. al., 2000) have also reported the derivation of human ES cells from human blastocyts. A third group (Shamblott et. al., 1998) have described EG cell derivation.

A feature of ES/EG cells is that, in the presence of fibroblast feeder layers, they retain the ability to divide in an undifferentiated state for several generations. If the feeder layers are removed then the cells differentiate. The differentiation is often to neurones or muscle cells but the exact mechanism by which this occurs and its control remain unsolved.

In addition to ES/EG cells a number of adult tissues contain cells with stem cell characteristics. Typically these cells, although retaining the ability to differentiate into different cell types, do not have the pluripotential characteristics of ES/EG cells. For example haemopoietic stem cells have the potential to form all the cells of the haemopoietic system (red blood cells, macrophages, basophils, eosinophils etc). All of nerve tissue, skin and muscle retain pools of cells with stem cell potential. Therefore, in addition to the use of embryonic stem cells in developmental biology, there are also adult stem cells which may also have utility with respect to determining the factors which govern cell differentiation. Further recent studies have suggested that some stem cells previously thought to be committed to a single fate, (e.g neurons) may indeed possess considerable pluripotentcy in certain situations. Neural

stem cells have recently been shown to chimerise a mouse embryo and form a wide range of non-neural tissue (Clark et. al., 2000).

A further group of cells which have relevance to developmental biology are teratocarcinoma cells (EC cells). These cells form tumours referred to as teratomas and have many features in common with ES/EG cells. The most important of these features is the characteristic of pluripotentiality.

Teratomas contain a wide range of differentiated tissues, and have been known in humans for many hundreds of years. They typically occur as gonadal tumours of both men and women. The gonadal forms of these tumours are generally believed to originate from germ cells, and the extra gonadal forms, which typically have the same range of tissues, are thought to arise from germ cells that have migrated incorrectly during embryogenesis. Teratomas are therefore generally classed as germ cell tumours which encompasses a number of different types of cancer. These include seminoma, embryonal carcinoma, yolk sac carcinoma and choriocarcinoma.

The similar biology of EC cells with ES/EG cells has been exploited to study the developmental fates of cells and to identify cell markers commonly expressed in EC cells and ES/EG cells. For example, and not by way of limitation, the expression of specific cell surface markers SSEA-3 (+), SSEA-4 (+), TRA-1-60 (+), TRA-1-81 (+) (Shevinsky *et al* 1982; Kannagi *et al* 1983; Andrews *et al* 1984a; Thomson *et al* 1995); alkaline phosphatase (+) (Andrews et. al., 1996); and Oct 4 (Scholer et. al., 1989; Kraft et. al., 1996; Reubinoff et. al., 2000; Yeom et. al., 1996).

It is well known that gene expression can be affected at many levels. For example, at the level of transcription, translation or post-translationally by modifications to proteins which confer an altered biological activity to the modified protein. It is also known that the way in which DNA is packaged as chromatin can influence the expression of genes.

There are several levels of structural packaging of DNA leading from a double stranded helix to a mitotic chromosome, after which the DNA is some ~50,000 times shorter than its extended length (Alberts *et al.*, 1998). Double-stranded helical DNA is wound around the structural unit of a nucleosome, comprising an octamer core composed of 4 types of histones: two each of the H2A, H2B, H3, and H4 proteins. Approximately 166 base pairs are bound to the nucleosome through electrostatic forces between the negatively charged phosphate groups in the DNA backbone and positively charged amino acids (e.g., lysine and arginine) in the histone proteins (Wolfe, 1993). Whilst the majority of the base pairs are tightly bound to the octamer core, the remaining linker DNA (80-100bp) hat separates adjacent core particles is associated with the H1 histone or a related "linker" histone (Finch and Klug, 1976; Thoma et al., 1979; Wolfe, 1993).

Nucleosomes are organised into the next structural level of the chromatin fibre, also referred to as a solenoid. Chromatin structure is not static and the regulated alteration in structure is termed 'chromatin remodelling'. This process has been defined as any event that alters the nuclease sensitivity of a region of chromatin, and can occur independently or in concert with processes such as transcription (Aalfs & Kingston, 2000). For a comprehensive review of chromatin remodelling see Aalfs & Kingston, 2000.

Reversible acetylation of evolutionary conserved lysine residues in core histone proteins plays a critical role in transcriptional regulation, cell cycle progression, and developmental events. The steady state of histone acetylation is controlled by the enzymatic activities of multiple histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone hyperacetylation is associated with transcriptional activity while histone hypoacetylation correlates with transcriptional quiescence and so histone deacetylases can be considered as enzymatic transcriptional repressors. Histone deacetylases were first described by Inove & Fujimoto, 1969.

In general, histone deacetylases do not target genes directly through specific DNA-binding sites. Rather, deacetylases are localized to genes targeted for repression as part of a protein complex. Other proteins that are part of this complex, termed co-repressors, are responsible for targeting the genes to be repressed. A large number of such co-repressors have been identified to date, including the thyroid hormone receptor, Sin3, SMRT, mYY1, and MeCP2, for a comprehensive review see Pazin & Kadonaga, 1997.

In humans, four highly homologous class I HDAC enzymes (HDAC1, HDAC2, HDAC3, and HDAC8) have been identified to date, with HDAC1, HDAC2 and HDAC3 being ubiquitously expressed in many different cell types (Yang *et al.*, 1997 and 2002). HDAC1 and HDAC2 are the human orthologues of the yeast transcriptional regulator RPD3. Analysis of the predicted amino acid sequence of HDAC3 revealed an open reading frame of 428 amino acids with a predicted molecular mass of 49 kDa.

The HDAC3 protein is 50% identical in DNA sequence and 53% identical in protein sequence compared with the previously cloned human HDAC1. Comparison of the HDAC3 sequence with human HDAC2 also yielded similar results, with 51% identity in DNA sequence and 52% identity in protein sequence (Yang *et al.*, 1997). The expressed HDAC3 protein is functionally active because it possesses histone deacetylase activity, represses transcription when tethered to a promoter, and binds transcription factor YY1. Although HDAC3 shares some structural and functional similarities with other class I HDACs, it exists in multi-subunit complexes separate and different from other known HDAC complexes, implying that individual HDACs might function in a distinct manner (Yang et al., 2002). Within the HDACs there are three regions of highly conserved amino acid residues; histidines, aspartates and glycines, irrespective of the highly divergent nature of the C-terminal regions (Hassig *et al.*, 1998). It is presumed that these regions form part of the active site and are also involved in maintaining interactions between HDACs and members of the co-repressor complex.

In *Drosophila*, active and silent states of developmentally regulated loci are maintained by *trithorax* and *Polycomb* group of proteins. Proteins of the polycomb and trithorax groups act to remodel chromatin by altering the accessibility of DNA to factors required for gene transcription. The PcG proteins are required to maintain the transcriptionally inactive state, whereas the trxG proteins are necessary to counteract silencing and maintain the transcriptionally active state. Both PcG and trxG proteins are thought to function by establishing closed or open chromatin configurations at their target genes

In humans, homologues of the members of the Drosopila polycomb group (Pc-G) proteins include; the YY1 transciption factor (YY1), the chromobox 2 gene (CBX1) and the PHD finger protein 1, transcript variant 2 (PHF1) gene. Pc-G proteins are usually considered to be inhibitors of homeotic genes. Pc-G mutants were originally identified on the basis of their causing expression of homeotic genes in unusual (ectopic) locations. This ectopic expression of genes was attributed to the failure of proper gene silencing. Pc-G proteins themselves are unable to bind to DNA, their action is dependent on their association with other chromosomal proteins, especially histones. It is suggested that the initial repression of a gene is carried out by transcription factors which have the ability to recognize DNA and that Pc-G proteins then provide a mechanism where this initial repression becomes permanent by assembling at this site and forming a multiprotein complex involved in modifying chromatin.

In humans, homologues of the members of the Drosophila Trithorax (TRX) proteins, for example an enhancer of polycomb 1 (EPC1), a zinc finger protein 144 (MEL18) and a myeloid/lymphoid or mixed lineage leukemia 1 (MLLT1) are considered to be activators of homeobox genes. Mutations within trx genes result in transformations of body structures reminiscent of loss-of-function mutations in homeotic genes. For example, in the Drospholia, after the disappearance of the transiently acting patterning factors such as those encoded by the segmentation genes, maintenance of

the initial transcriptional patterns of homeotic genes requires the expression of the trx gene. (Orlando *et al.*, 1998). Genetic analyses indicate that *trx* expression is required continuously throughout Drosophila development, consistent with its maintenance function, but there also appears to be a critical early requirement, which if compromised cannot be compensated by subsequent continuous expression (Ingham and Whittle, 1980). Comparatively little is known about the molecular environment in which the TRX protein is integrated. However, an elucidation of this issue could be particularly rewarding as chromosomal aberrations involving the human homologues of TRX (MLL, ALL-1, HRX) is one of the most frequent genetic changes in infant leukemias of myeloid and lymphoid lineage and in treatment-induced secondary leukemias (Orlando *et al.*, 1998).

Whereas some multi-protein complexes which alter transcriptional regulation and chromatin remodelling are based on the covalent modification of the histones (e.g. histone acetyltransferases (HATs) and histone deacetylases, others appear to function by altering chromatin structure in an ATP dependent fashion (e.g. the yeast SWI/SNF complex). A group of enzymes referred to as ATP-dependent chromatin remodellers, use the energy of ATP hydrolysis to alter interactions between DNA and histone proteins. The protein complexes that mediate ATP-dependent nucleosome remodelling and histone acetylation/deacetylation in the regulation of transcription were initially considered to be different, although it has recently been suggested that their activities might be coupled. Examples of human ATP-dependent chromatin remodellers include, SMARCA5, a human SWI/SNF related, matrix associated and actin dependent regulator of chromatin, identified as member 5 of subfamily 'a' of the SMARC family.

There are further examples of regulators of gene expression. We have accumulated expression studies which identify a number of genes thought to be involved in determining the developmental fate of stem cells, particularly embryonic stem cells. By northern blotting we have identified the expression of human homologs of two signalling pathways believed to be critical in cell fate determination. Expression of

ligands, receptors and downstream components of the Notch and Wingless signalling cascades have been elucidated. Using the model system NTERA2/D1 embryonal carcinoma cells we have recorded changes in the expression of some of these components as the cells differentiate. Bearing in mind the role these cascades play in embryonic development throughout the animal kingdom, these changes suggest a significant role for both the wingless and Notch signalling pathways in differentiation of stem cells. Furthermore the activity of some genes are required for differentiation to occur along specific pathways e.g. the myogenic gene MyoD1. Other genes have activity which inhibits cellular differentiation along particular pathways. We envisage regulation of stem cell differentiation to yield a specific cell type could be achieved by:

(i) inhibition of certain genes that normally promote differentiation along particular pathways; therefore promoting differentiation to alternate cell phenotypes;

(ii) inhibition of gene activity that prevents differentiation into particular cell types; and

(iii) a combination of (i) and (ii), see figure 1

The differentiation of stem cells during embryogenesis, during tissue renewal in the adult and wound repair is under very stringent regulation: aberrations in this regulation underlie the formation of birth defects during development and are thought to underlie cancer formation in adults. Generally, it is envisaged that such stem cells are under both positive and negative regulation which allows a fine degree of control over the process of cell proliferation and cell differentiation: excess proliferation at the expense of cell differentiation can lead to the formation of an expanding mass of tissue – a cancer – whereas express differentiation at the expense of proliferation can lead to the loss of stem cells and production of too little differentiated tissue in the long term, and especially the loss of regenerative potential. Certain genes have already been identified to have a negative role in preventing stem cell differentiation. Such genes, like those of the Notch family, when mutated to acquire activity can

inhibit differentiation; such mutant genes act as oncogenes. On the contrary, loss of function of such genes on their inhibition results in stem cell differentiation. We propose to use EC cells as our model cell system to follow the effects of RNAi on cell fate.

In our co-pending application, WO02/16620, discloses RNAi molecules derived from the following nucleic acid sequences which encode the following polypeptides; human Notch 1(hNotch); hNotch 2; hNotch 3; hNotch 4; TLE-1; TLE-2; TLE-3; TLE-4; TCF7; TCF7L1; TCFFL2; TCF3; TCF19; TCF1; mFringe; lFringe; rFringe; sel 1; Numb; Numblike; LNX; FZD1; FZD2; FZD3; FZD4; FZD5; FZD6; FZD7; FZD8; FZD9; FZD10; FRZB, D11-1; D113; D114; Dlk-1; Jagged 1; Jagged 2; Wnt 1; Wnt 2; Wnt 2b; Wnt 3; Wnt 3a; Wnt5a; Wnt6; Wnt7a; Wnt7b; Wnt8a; Wnt8b; Wnt10b; Wnt11; Wnt14; Wnt15, SFRP1; SFRP2; SFRP4; SFRP5; SK; DKK3; CER1; WIF-1; DVL1; DVL2; DVL3; DVL1L1;mFringe; lFringe; rFringe; sel1l; Numb; LNX Oct4; NeuroD1; NeuroD2; NeuroD3; Brachyury; MDFI, CIR, DLK1; Oct 4; RBPJk. The present application disclaims these genes the sequences of which are disclosed in WO02/16620.

One further family of genes are the HES and related genes which are direct targets of Notch signaling. Binding of Notch ligands to Notch receptor causes proteolytic cleavage of the receptor (Mumm and Kopan, 2000). The cleaved receptor known as Notch-intracellular domain (NICD) translocates to the nucleus and binds to RBP-Jκ. This binding changes RBP-Jκ from a repressor to an activator of its target genes. The target genes are homologs of the genes found at the Drosophila Enhancer of Split complex (E(spl)). These basic helix-loop-helix (bHLH) transcription factors act as repressors of downstream tissue specific transcription factors and as such act as notch effectors. The Notch signaling through E(spl) complex genes represses certain tissue specific transcription factors.

The E(spl) family of proteins are class three bHLH factors. These include: HES1, HES2, HES4, HES6, HES7, HERP1, HERP2, HESR1, HEY1, HEY2, HEYL HRT1, HRT2, HRT3 CHF1, CHF2 GRIDLOCK.

The various members of the HES related genes encode proteins that are homologous in key motif regions. They all contain Basic helix-loop-helix and a so called orange domain. HES family members contain a terminal WRPW domain and HEY family proteins contain YRPW or closely related residues. Figure 1 shows an alignment of human HES related proteins illustrating the major domains contained in the HES related proteins. ES/EC differentiation go through a precursor stage for example neural differentiation (Przyborski et. al., 2001) during differentiation to the numerous lineages that can form *in vitro*.

Notch signaling through E(spl) homologs possibly allows precursor cells to remain as precursors. In addition Notch may also play an instructive role in specifying cell types. for example (Hojo et. al., 2000). Manipulation of the E(spl) homologs and other downstream targets which directly affect these processes would alter the notch signaling in target cells. This in turn would alter the balance between cells types. This could be manipulated to for example block a particular cell type forming by stopping the instructive signaling or by increasing or removing the precursor cells from the cultures. The E(spl) complex genes are potential targets which would allow cell type specific disruption of Notch signaling in differentiating cultures of stem cells.

According to an aspect of the invention there is provided a method to modulate the differentiation state of a stem cell comprising the steps of:

i) contacting a stem cell with at least one inhibitory RNA molecule (RNAi) comprising a sequence of a gene which mediates at least one step in the differentiation of said cell;

(ii) providing conditions conducive to the proliferation of the cell treated in (i) above; and optionally

(iii) maintaining and/or storing said cell.

The term modulate includes both promoting or inducing the differentiation of a stem cell into a lineage restricted stem cell or a differentiated cell or to maintain a stem

cell as a stem cell with characteristics which are typical of stem cells, particularly embryonic stem cells. For example, maintenance in culture for at least 20 passages when maintained on fibroblast feeder layers; production of embryoid bodies in culture; the ability to differentiate into multiple cell types in monolayer culture; can form embryo chimeras when mixed with an embryo host; and express ES/EG cell specific markers.

In a preferred method of the invention said method is an *in vitro* method.

In an alternative preferred method said method is an *in vivo* method.

In a further preferred method of the invention said stem cell is selected from the group consisting of: haemopoietic stem cells; neural stem cells; bone stem cells; muscle stem cells; mesenchymal stem cells; trophoblastic stem cells; epithelial stem cells (derived from organs such as the skin, gastrointestinal mucosa, kidney, bladder, mammary glands, uterus, prostate and endocrine glands such as the pituitary); endodermal stem cells (derived from organs such as the liver, pancreas, lung and blood vessels); embryonic stem (ES) cells; embryonal germ (EG) cells.

In a further preferred method of the invention said stem cells are embryonal carcinoma cells. Preferably said embryonal carcinoma cells are TERA2 cells. Ideally said embyonal carcinoma cells are NTERA 2 cells.

In a further preferred method of the invention said stem cell is an embryonic stem cell or embryonal germ cell or an embryonal carcinoma cell.

In a preferred method of the invention said gene is involved in Notch/Wnt signalling.

In a preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of:

i) a nucleic acid sequence as represented by table 1, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequence of table 1 and which modulates stem cell differentiation;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a preferred method of the invention said hybridisation conditions are stringent hybridisation conditions.

Typically, hybridisation conditions uses 4 – 6 x SSPE (20xSSPE contains 175.3g NaCl, 88.2g NaH_2PO_4 H_2O and 7.4g EDTA dissolved to 1 litre and the pH adjusted to 7.4); 5-10x Denhardts solution (50x Denhardts solution contains 5g Ficoll (type 400, Pharmacia), 5g polyvinylpyrrolidone abd 5g bovine serum albumen; 100µg-1.0mg/ml sonicated salmon/herring DNA; 0.1-1.0% sodium dodecyl sulphate; optionally 40-60% deionised formamide. Hybridisation temperature will vary depending on the GC content of the nucleic acid target sequence but will typically be between 42^0- 65^0 . It is well known in the art that optimal hybridisation conditions can be calculated if the sequences of the nucleic acid is known. For example, hybridisation conditions can be determined by the GC content of the nucleic acid subject to hybridisation. Please see Sambrook *et al* (1989) Molecular Cloning; A Laboratory Approach. A common formula for calculating the stringency conditions required to achieve hybridisation between nucleic acid molecules of a specified homology is:

$T_m = 81.5^0\,C + 16.6\,\mathrm{Log}\,[Na^+] + 0.41[\,\%\,G + C] - 0.63$ (%formamide).

In a preferred method of the invention said RNAi molecule is derived from a nucleic acid sequence encoding a Notch receptor processing factor polypeptide selected from the group consisting of: Nrarp; P300; presenilin associated protein; presenilin 1; presenilin 2; or Sel-1.

In an alternative preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a Notch target gene selected from the group consisting of: HERP1; HERP2; HES1; HES 2; HES 4; HES6, HES7, HERP1, HERP2, HESR1, HEY1, HEY2, HEYL HRT1, HRT2, HRT3 CHF1, CHF2 GRIDLOCK.

In a preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in SEQ ID NO: 7-23, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of SEQ ID NO: 7-23 and is a Notch-signalling target gene;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a further alternative preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt ligand processing factor selected from the group consisting of: LRP1; LRP2; LRP3; LRP4; LRP5; LRP6; LRP8; or Porcupine.

Alternatively said RNAi molecule is derived from a nucleic acid molecule encoding an extracellular Wnt antagonist selected from the group consisting of: Dkk1; Dkk2; Dkk3; Dkk4; Frzb; or SARP1.

In a further preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt cytoplasmic acting component selected from the group consisting of: APC; Axin1; Axin2; FRAT1; GSK3; ICAT; IDAX; Par 1; or TAB1.

Alternatively, said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt nuclear acting component selected from the group consisting of: β-catenin; β-TRCP; CBP; CTBP1; HBP-1; Lef1; NLK; Pontin 52; Reptin 52.

In a yet further alternative method of the invention said RNAi molecule is derived from a nucleic acid molecule which encodes a Wnt target gene selected from ASCL 1 or ASCL 2.

In a yet still further preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule selected from the group consisting of: FGF 5; msx 1; neurogenin 1; neurogenin 2; neurogenin 3; or PTEN.

In an alternative preferred method of the invention said RNAi molecule is derived from a gene which encodes a polypeptide involved in modifying chromatin conformation.

In a preferred method of the invention said RNAi molecule is derived from a nucleic acid sequence which encodes a polypeptide which modifies a histone polypeptide. Preferably said histone modifying polypeptide is a histone deacetylase. Preferably said RNAi is derived from a mammalian class I histone deacetylase.

In a preferred method of the invention said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in Table 4, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of Table 4 and which has histone deacetylase activity;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

Preferably said histone deacetylase is selected from the group consisting of: HDAC1; HDAC2; HDAC3; HDAC 4; HDAC5; HDAC6; HDAC7; HDAC8; hSIRT2; hSIRT3; hSIRT4; hSIRT5; hSIRT6; hSIRT7; MECP2; ZNF145; TFDP1; SAP30; SAP18; RBBP7; RBBP4; RB1;MEN1.

Alternatively, said histone modifying polypeptide is a histone acetyltransferase selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in Table 5, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of Table 5 and which has histone acetyltransferase activity;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a preferred method of the invention said histone acetyltransferase is selected from the group consisting of: Gcn 5; Gcn5L2; PCAF; MOZ; HBO; CBP; SCR-1; pGRIP; ATF-2; and HAT1.

In a further preferred method of the invention said RNAi molecule comprises a nucleic acid sequence derived from a gene selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in Table 2, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of Table 2 and which mediates chromatin conformation;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a preferred method of the invention said nucleic acid encodes a polypeptide which mediates chromatin conformation selected from the group consisting of: EED; YY1;

CBX1; CBX6; HPC2(CBX4); HPC3(CBX8); PHF1; PHF2; HPH1; HPH2; SSX1; and SSX2.

In a further preferred method of the invention said RNAi molecule comprises a nucleic acid sequence derived from a gene selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in Table 3, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of Table 3 and which mediates chromatin conformation;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a preferred method of the invention said nucleic acid encodes a polypeptide which mediates chromatin conformation selected from the group consisting of: EPC1; EZH1; EZH2; BMI1; MEL18; SCML1; SCML2; RING1; RYBP; MLL; MLLT1; MLLT7; MLLT6; MLLT4; MLLT3; MLLT2; MLLT10; and MLL2.

In a further preferred method of the invention said RNAi molecule comprises a nucleic acid sequence derived from a gene selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in Table 6, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of Table 6 and which mediates chromatin conformation;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a preferred method of the invention said nucleic acid encodes a polypeptide which mediates chromatin conformation selected from the group consisting of: SMARCA

5; SMARCA 2; SMARCA 4; SMARCA 3; SMARCAL1; SMARCA 1; and CHRAC1.

In a preferred method of the invention said RNAi molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their length and further wherein said first and second parts form a double stranded region by complementary base pairing over at least part of their length.

The provision of first and second sequences which are complementary to one another and which comprise at least part of the coding sequence of a gene involved in stem cell differentiation means that when the sequence is transcribed into RNA the complementarity between first and second sequences allows base pairing between first and second sequences to form a double stranded RNA structure. The optional provision of a linking region bewteen first and second parts results in the formation of a so called "hair-pin" loop structure. The transcription of the nucleic acid provides many copies of the hair-pin loop RNA which effectively functions as a RNAi molecule. The hair-pin loop RNA can be transcribed *in vitro* using, for example commercially available transcription kits which utilise phage RNA polymerase or *in vivo* using vectors adapted for expression by a cell, typically a eukaryotic cell, preferably a lineage restricted stem cell or embryonic stem cell.

According to a further aspect of the invention there is provided an RNAi molecule which comprises a sequence of a gene wherein said gene mediates stem cell differentiation.

In a preferred embodiment of the invention said RNAi molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their length and further wherein said first and second parts form a double stranded region by complementary base pairing over at least part of their length.

In a further preferred embodiment of the invention said first and second parts are linked by at least one nucleotide base. In a further preferred embodiment of the invention said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide bases. In a yet further preferred embodiment of the invention said linker is at least 10 nucleotide bases.

In a further preferred embodiment said coding sequence is an exon.

Alternatively said RNA molecule is derived from intronic sequences or the 5' and/or 3' non-coding sequences which flank coding/exon sequences of genes which modulate stem cell differentiation.

In a further preferred embodiment of the invention the length of the RNAi molecule is between 10 nucleotide bases (nb) –1000nb. More preferably still the length of the RNA molecule is selected from 10nb; 20nb; 30nb; 40nb; 50nb; 60nb; 70nb; 80nb; 90nb. More preferably still said RNA molecule is 21nb in length. Preferably said RNAi molecule comprises 19 complementary bases with a 3' 2nb overhang at either end.

In a further preferred embodiment of the invention said RNA molecule is 100nb; 200nb; 300nb; 400nb; 500nb; 600nb; 700nb; 800nb; 900nb; or 1000nb. More preferably still said RNA molecule is at least 1000nb.

In yet a further preferred embodiment of the invention said RNAi molecules comprise modified nucleotide bases.

It will be apparent to one skilled in the art that the inclusion of modified bases, as well as the naturally occuring bases cytosine, uracil, adenosine and guanosine, may confer advantageous properties on RNAi molecules containing said modified bases. For example, modified bases may increase the stability of the RNAi molecule thereby

reducing the amount required to produce a desired effect. The provision of modified bases may also provide RNAi molecules which are more or less stable.

The term "modified nucleotide base" encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleotides may also include 2' substituted sugars such as 2'-O-methyl-; 2-O-alkyl; 2-O-allyl; 2'-S-alkyl; 2'-S-allyl; 2'- fluoro-; 2'-halo or 2;azido-ribose, carbocyclic sugar analogues a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and sedoheptulose.

Modified nucleotides are known in the art and include by example and not by way of limitation; alkylated purines and/or pyrimidines; acylated purines and/or pyrimidines; or other heterocycles. These classes of pyrimidines and purines are known in the art and include, pseudoisocytosine; N4, N4-ethanocytosine; 8-hydroxy-N6-methyladenine; 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil; 5-fluorouracil; 5-bromouracil; 5-carboxymethylaminomethyl-2-thiouracil; 5-carboxymethylaminomethyl uracil; dihydrouracil; inosine; N6-isopentyl-adenine; 1-methyladenine; 1-methylpseudouracil; 1-methylguanine; 2,2-dimethylguanine; 2-methyladenine; 2-methylguanine; 3-methylcytosine; 5-methylcytosine; N6-methyladenine; 7-methylguanine; 5- methylaminomethyl uracil; 5-methoxy amino methyl-2-thiouracil; β-D-mannosylqueosine; 5-methoxycarbonylmethyluracil; 5-methoxyuracil; 2 methylthio-N6-isopentenyladenine; uracil-5-oxyacetic acid methyl ester; psueouracil; 2-thiocytosine; 5-methyl-2 thiouracil, 2-thiouracil; 4-thiouracil; 5-methyluracil; N-uracil-5-oxyacetic acid methylester; uracil 5—oxyacetic acid; queosine; 2-thiocytosine; 5-propyluracil; 5-propylcytosine; 5-ethyluracil; 5-ethylcytosine; 5-butyluracil; 5-pentyluracil; 5-pentylcytosine; and 2,6,-diaminopurine; methylpsuedouracil; 1-methylguanine; 1-methylcytosine;

The RNAi molecules of the invention can be synthesized using conventional phosphodiester linked nucleotides and synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may use alternative linking molecules. For example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through –O- or –S-.

According to a further aspect of the invention there is provided a nucleic acid molecule encoding at least part of a gene which modulates stem cell differentiation comprising a first part linked to a second part which first and second parts are complementary over at least part of their length, wherein said nucleic acid molecule is operably linked to at least one further nucleic acid molecule capable of promoting transcription of said nucleic acid linked thereto and further wherein said first and second parts form a double stranded region by complementary base pairing over at least part of their length as or when said nucleic acid molecule is transcribed.

In a preferred embodiment of the invention said first and second parts are linked by linking nucleotides as hereinbefore described.

It will be apparent to one skilled in the art that the synthesis of RNA molecules which form RNA stem loops can be achieved by providing vectors which include target genes, or fragments of target genes, operably linked to promoter sequences. Typically, promoter sequences are phage RNA polymerase promoters (eg T7, T3, SP6). Advantageously vectors are provided with multiple cloning sites into which genes or gene fragments can be subcloned. Typically, vectors are engineered so that phage promoters flank multiple cloning sites containing the gene of interest.

Alternatively target genes or fragments of target genes can be fused directly to phage promoters by creating chimeric promoter/gene fusions via oligo synthesising technology. Constructs thus created can be easily amplified by polymerase chain

reaction to provide templates for the manufacture of RNA molecules comprising stem loop RNA's.

According to a further aspect of the invention there is provided an expression vector including an expression cassette comprising at least one nucleic acid molecule encoding an RNAi molecule according to the invention.

Vectors including expression cassettes encoding stem-loop RNA's are adapted for eukaryotic gene expression. Typically said adaptation includes, by example and not by way of limitation, the provision of transcription control sequences (promoter sequences) which mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific, inducible or constitutive.

Promoter elements typically also include so called TATA box and RNA polymerase initiation selection sequences which function to select a site of transcription initiation. These sequences also bind polypeptides which function, *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers and autonomous replication sequences which both facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic host. Vectors which are maintained autonomously are referred to as episomal vectors. Further adaptations which facilitate the expression of vector encoded genes include the provision of transcription termination sequences.

These adaptations are well known in the art. There is a significant amount of published literature with respect to expression vector construction and recombinant DNA techniques in general. Please see, Sambrook et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) DNA Cloning Techniques: A Practical

Approach Vol III IRL Press, Oxford UK; DNA Cloning: F M Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, Inc.(1994).

In a preferred embodiment of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a notch receptor processing factor polypeptide selected from the group consisting of: Nrarp; P300; presenilin associated protein; presenilin 1; presenilin 2; or Sel-1.

In an alternative preferred embodiment said RNAi molecule is derived from a nucleic acid molecule encoding a Notch target gene selected from the group consisting of: HERP1; HERP2; HES1; HES 2; HES 4; HES6, HES7, HERP1, HERP2, HESR1, HEY1, HEY2, HEYL HRT1, HRT2, HRT3 CHF1, CHF2 GRIDLOCK.

In a preferred embodiment of the invention said RNAi molecule is derived from a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of:

i) a nucleic acid sequence as represented by the sequences in SEQ ID NO: 7-23, or fragment thereof;

ii) a nucleic acid sequence which hybridises to the nucleic acid sequences of SEQ ID NO: 7-23 and is a Notch signalling target gene;

iii) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

In a further alternative method of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt ligand processing factor selected from the group consisting of: LRP1; LRP2; LRP3; LRP4; LRP5; LRP6; LRP8; or Porcupine.

In a yet further alternative method said RNAi molecule is derived from a nucleic acid molecule encoding an extracellular Wnt antagonist selected from the group consisting of: Dkk1; Dkk2; Dkk3; Dkk4; Frzb; or SARP1.

In a further preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt cytoplasmic acting component selected from the group consisting of: APC; Axin1; Axin2; FRAT1; GSK3; ICAT; IDAX; Par 1; or TAB1.

Alternatively, said RNAi molecule is derived from a nucleic acid molecule encoding a Wnt nuclear acting component selected from the group consisting of: β-catenin; β-TRCP; CBP; CTBP1; HBP-1; Lef1; NLK; Pontin 52; or Reptin 52.

In a yet further preferred method of the invention said RNAi molecule is derived from a nucleic acid molecule which encodes a Wnt target gene selected from ASCL 1 or ASCL 2.

In a yet still further preferred method of the invention said RNAi molecule is derived from the group consisting of: FGF 5; msx 1; neurogenin 1; neurogenin 2; neurogenin 3 ; or PTEN.

According to a further aspect of the invention there is provided a method of treatment of an animal, preferably a human, comprising administering an effective amount of at least one RNAi molecule according to the invention, to a subject to be treated.

According to a yet further aspect of the invention there is provided a method of treatment of an animal, preferably a human, comprising administering an effective amount of at least one vector which includes an RNAi molecule according to the invention, to a subject to be treated.

An effective amount is an amount sufficient to induce the differentiation of at least one stem cell into at least one lineage restricted stem cell or differentiated stem cell.

According to a further aspect of the invention there is provided a lineage restricted stem cell or a differentiated stem cell obtainable by the method according to the invention.

In a preferred embodiment of the invention said lineage restricted stem cell is selected from the group consisting of: haemopoietic stem cell; neural stem cell; bone stem cell; muscle stem cell; mesenchymal stem cell; trophoblastic stem cell; epithelial stem cell (derived from organs such as the skin, gastrointestinal mucosa, kidney, bladder, mammary glands, uterus, prostate and endocrine glands such as the pituitary); endodermal stem cell (derived from organs such as the liver, pancreas, lung and blood vessels).

In a further preferred embodiment of the invention said cell is selected from the group consisting of: a nerve cell; a mesenchymal cell; a muscle cell (cardiomyocyte); a liver cell; a kidney cell; a blood cell (eg erythrocyte, CD4+ lymphocyte, CD8+ lymphocyte; panceatic β cell; epithelial cell (eg lung, gastric,) ; an endothelial cell.

According to a yet further aspect of the invention there is provided a cell culture comprising at least one lineage restricted stem cell or differentiated cell according to the invention.

According to a further aspect of the invention there is provided an organ comprising a lineage restricted stem cell or a differentiated stem cell according to the invention.

According to a yet further aspect of the invention there is provided a method of treatment of an animal, preferably a human, comprising administering a cell or organ according to the invention.

An embodiment of the invention will now be described by example only and with reference to the following figures and tables wherein:

Table 1 represents the nucleic acid sequences of Notch/Wnt target genes molecules from which RNAi molecules are derived;

Table 2 represents nucleic acid sequences of polycomb target genes from which RNAi molecules are derived;

Table 3 represents nucleic acid sequences of enhancers of trithorax and polycomb target genes from which RNAi molecules are derived;

Table 4 represents nucleic acid sequences of histone deacetylase target genes from which RNAi molecules are derived;

Table 5 represents nucleic acid sequences of histone acetylase target genes from which RNAi molecules are derived;

Table 6 represents nucleic acid sequences of ATP dependent chromatin modification target genes from which RNAi molecules are derived;

Table 7 represents a selection of antibodies used to monitor stem cell differentiation;

Table 8 represents nucleic acid probes used to assess mRNA markers of stem differentiation;

Table 9 represents protein markers of stem cell differentiation;

Figure 1 illustrates stem cell differentiation is controlled by positive and negative regulators (A). The specific cell phenotypes that are derived are a direct result of positive and negative regulators which activate or suppress particular differentiation events. RNAi can be used to control both the initial differentiation of stem cells (A) and the ultimate fate of the differentiated cells D1 and D2 by repression of positive activators which would normally promote a particular cell fate;

Figure 2 represents (A) a schematic diagram illustrating the Notch and Wnt signalling pathways. The Notch and Wnt signaling pathways are shown.

Materials and Methods

Cell Culture

NTERA2 and 2102Ep human EC cell lines were maintained at high cell density as previously described (Andrews et al 1982, 1984b), in DMEM (high glucose formulation) (DMEM)(GIBCO BRL), supplemented with 10% v/v bovine foetal calf serum (GIBCO BRL), under a humidified atmosphere with 10% CO_2 in air.

Double stranded RNA synthesis

PCR primers were designed against the mRNA sequence of interest to give a product size of around 500bp. At the 5' end of each primer was added a T7 RNA polymerase promoter, comprising one or other of the following sequences: TAATACGACTCACTATAGGG; AATTATAATACGACTCACTATA. PCR was performed using these primers on an appropriate cDNA source (e.g. derived from the cell type to be targeted) and the product cloned and sequenced to confirm its identity. Using the sequenced clone as a template, further PCRs were performed as required to generate template DNA for RNA synthesis. In each case, a quantity of the PCR was electrophoresed through agarose to verify product size and abundance, whilst the remainder was purified by alkaline phenol/chloroform extraction. RNA was synthesized using the Megascript kit (Ambion Inc.) according to the manufacturer's protocol and acid phenol/chloroform extracted. The simultaneous synthesis of complementary strands of RNA in a single reaction circumvents the requirement for an annealing step. However, the quality and duplexing of the synthesized RNA was confirmed by agarose gel electrophoresis, with the desired products migrating as expected for double stranded DNA of the same length.

Treatment of human cells with dsRNA to produce RNAi

The following method describes RNAi of cells cultured in 6 well plates. Volumes and cell numbers should be scaled appropriately for larger or smaller culture vessels.

Cells were seeded at 500,000 per well on the day prior to treatment and grown in their normal medium. For each well to be treated, 9.5µg of the double stranded RNA of interest was diluted in 300µl of 150mM NaCl. 21µl of ExGen 500 (MBI Fermentas) was added to the diluted RNA solution and mixed by vortexing. The dsRNA/ExGen 500 mixture was incubated at room temperature for 10 minutes. 3ml of fresh cell growth medium was then added, producing the RNAi treatment medium. Growth medium was aspirated from the culture vessel and replaced with 3ml of RNAi treatment medium per well. Culture vessels were then centrifuged at 280g for 5 minutes and returned to the incubator. After 12-18hrs, RNAi treatment medium was replaced with normal growth medium and the cells maintained as required.

Total RNA production

Growing cultures of cells were aspirated to remove the DME and foetal calf serum. Trace amounts of foetal calf serum was removed by washing in Phosphate-buffered saline. Fresh PBS was added to the cells and the cells were dislodged from the culture vessel using acid washed glass beads. The resulting cell suspension was centrifuged at 300xg. The pellets had the PBS aspirated from them. Tri reagent (Sigma, USA) was added at 1ml per 10^7 cells and allowed to stand for 10 mins at room temperature. The lysate from this reaction was centrifuged at 12000 x g for 15 minutes at 4°C. The resulting aqueous phase was transferred to a fresh vessel and 0.5 ml of isopropanol / ml of trizol was added to precipitate the RNA. The RNA was pelleted by centrifugation at 12000 x g for 10 mins at 4°C. The supernatant was removed and the pellet washed in 70% ethanol. The washed RNA was dissolved in DEPC treated double-distilled water.

Analysis of the differentiation of EC stem cells induced by exposure to RNAi

Following exposure to RNAi corresponding to specific key regulatory genes, the subsequent differentiation of the EC cells was monitored in a variety of ways. One approach was to monitor the disappearance of typical markers of the stem cell phenotype; the other was to monitor the appearance of markers pertinent to the specific lineages induced. The relevant markers included surface antigens, mRNA species and specific proteins.

Analysis of Transfectants by Antibody Staining and FACS

Cells were treated with trypsin (0.25% v/v) for 5 mins to disaggregate the cells; they were washed and re-suspended to $2x10^5$ cells/ml. This cell suspension was incubated with 50µl of primary antibody in a 96 well plate on a rotary shaker for 1 hour at 4°C. Supernatant from a myeloma cell line P3X63Ag8, was used as a negative control. The 96 well plate was centrifuged at 100rpm for 3 minutes. The plate was washed 3 times with PBS containing 5% foetal calf serum to remove unbound antibody. Cell were then incubated with 50 µl of an appropriate FITC-conjugated secondary antibody at 4°C for 1 hour. Cells were washed 3 times in PBS + 5% foetal calf serum and analysed using an EPICS elite ESP flow cytometer (Coulter eletronics, U.K).(Andrews et. al., 1982)

Northern blot Analysis of RNA

RNA separation relies on the generally the same principles as standard DNA but with some concessions to the tendency of RNA to hybridise with itself or other RNA molecules. Formaldehyde is used in the gel matrix to react with the amine groups of the RNA and form Schiff bases. Purified RNA is run out using standard agarose gel electrophoresis. For most RNA a 1% agarose gel is sufficient. The agarose is made in 1X MOPS buffer and supplemeted with 0.66M formaldehyde.Dryed down RNA samples are reconstituted and denatured in RNA loading buffer and loaded into the

gel. Gels are run out for apprx. 3 hrs (until the dye front is 3/4 of the way down the gel).

The major problem with obtaining clean blotting using RNA is the presence of formaldehyde. The run out gel was soaked in distilled water for 20 mins with 4 changes, to remove the formaldehyde from the matrix. The transfer assembly was assembled in exactly the same fashion as for DNA (Southern) blotting.The transfer buffer used however was 10X SSPE. Gels were transfered overnight. The membrane was soaked in 2X SSPE to remove any agarose from the transfer assembly and the RNA was fixed to the memebrane. Fixation was acheived using short-wave (254 nM) UV light. The fixed membrane was baked for 1-2 hrs to drive off any residual formaldehyde.

Hybridisation was acheived in aqueous phase with formamide to lower the hybridisation temperatures for a given probe. RNA blots were prehybridised for 2-4 hrs in northern prehybridisation soloution. Labelled DNA probes were denatured at 95°C for 5 mins and added to the blots. All hybridisation steps were carried out in rolling bottles in incubation ovens. Probes were hybridised overnight for at least 16 hrs in the prehybridisation soloution. A standard set of wash soloutions were used. Stringency of washing was acheived by the use of lower salt containing wash buffers. The following wash procedure is outlined as follows

2X SSPE	15 mins	room temp
2X SSPE	15 mins	room temp
2X SSPE/ 0.1% SDS	45 mins	65°C
2X SSPE/ 0.1% SDS	45 mins	65°C
0.1X SSPE	15 mins	room temp

Preparation of radiolabelled DNA probes

The method of Feinberg and Vogelstein (Feinberg and Vogelstein, 1983) was used to radioactively label DNA. Briefly, the protocol uses random sequence hexanucleotides to prime DNA synthesis at numerous sites on a denatured DNA template using the

Klenow DNA polymerase I fragment. Pre-formed kits were used to aid consistency . 5-100ng DNA fragment (obtained from gel purifcation of PCR or restriction digests) was made up in water,denatured for 5 mins at 95°C with the random hexamers. The mixture was quench cooled on ice and the following were added,

5 µl [α-32P] dATP 3000 Ci/mmol

1 µl of Klenow DNA polymerase (4U)

The reaction was then incubated at 37°C for 1 hr. Unincorporated nucleotide were removed with spin columns (Nucleon Biosciences).

Production of cDNA

The enzymatic conversion of RNA into single stranded cDNA was achieved using the 3' to 5' polymerase activity of recombinant Moloney-Murine Leukemia Virus (M-MLV) reverse transcriptase primed with oligo (dT) and (dN) primers. For Reverse Transcription-Polymerase Chain Reaction, single stranded cDNA was used. cDNA was synthesised from 1µg poly (A)+ RNA or total RNA was incubated with the following

1.0µM	oligo(dT) primer for total RNA or random hexcamers for mRNA
0.5mM	10mM dNTP mix
1U/µl	RNAse inhibitor (Promega)
1.0U/µl	M-MLV reverse transcriptase in manufacturers supplied buffer (Promega)

The reaction was incubated for 2-3 hours at 42°C

Fluorescent Automated Sequencing

To check the specificity of the PCR primers used to generate the template used in RNAi production automatic sequencing was carried out using the prism fluorescently labelled chain terminator sequencing kit (Perkin-Elmer) (Prober et al 1987). A suitable amount of template (200ng plasmid, 100ng PCR product), 10 µM sequencing primer (typically a 20mer with 50% G-C content) were added to 8 µl of

prism pre-mix and the total reaction volume made up to 20 µl. 24 cycles of PCR (94°C for 10 seconds, 50°C for 10 seconds, 60°C for 4 minutes). Following thermal cycling, products were precipitated by the addition of 2µl of 3M sodium acetate and 50 µl of 100 % ethanol. DNA was pelleted in an Eppendorf microcentrifuge at 13000 rpm, washed once in 70% ethanol and vacuum dried. Samples were analysed by the in-house sequencing Service (Krebs Institute). Dried down samples were resuspended in 4 µl of formamide loading buffer, denatured and loaded onto a ABI 373 automatic sequencer. Raw sequence was collected and analysed using the ABI prism software and the results were supplied in the form of analysed histogram traces.

Detection of specific protein targets by SDS-PAGE and Western Blotting

To obtain cell lysates monolayers of cells were rinsed 3 times with ice-cold PBS supplemented with 2 mM $CaCl_2$. Cells were incubated with 1 ml/75 cm^2 flask lysis buffer (1% v/v NP40, 1% v/v DOC, 0.1 mM PMSF in PBS) for 15 min at 4°C. Cell lysates were transferred to eppendorf tubes and passed through a 21 gauge needle to shear the DNA. This was followed by freeze thawing and subsequent centrifugation (30 min, 4°C, 15000*g*) to remove insoluble material. Protein concentrations of the supernatants were determined using a commercial protein assay (Biorad) and were adjusted to 1.3 mg/ml. Samples were prepared for SDS-PAGE by adding 4 times Laemmli electrophoresis sample buffer and boiling for 5 min. After electrophoresis with 16 µg of protein on a 10% polyacrylamide gel (Laemmli, 1970) the proteins were transferred to nitro-cellulose membrane with a pore size of 0.45µm. The blots were washed with PBS and 0.05% Tween (PBS-T). Blocking of the blots occurred in 5% milk powder in PBS-T (60 min, at RT). Blots were incubated with the appropriate primary antibody. Horseradish peroxidase labelled secondary antibody was used to visualise antibody binding by ECL (Amersham, Bucks., UK). Materials used for SDS-PAGE and western blotting were obtained from Biorad (California, USA) unless stated otherwise.

Table7: Antibodies used to detect stem cell differentiation

Antibody	Class	Species	Cell phenotype detected	Changes on Differentiation	Reference
TRA-1-60	IgM	Mouse	Human EC, ES cells.	↓ differentiation	Andrews et.al., 1984a
TRA-1-81	IgM	Mouse	Human EC, ES cells.	↓ differentiation	Andrews et. al.,1984a
SSEA3	IgM	Rat	Human EC, ES cells.	↓ differentiation	Shevinsky et al 1982, Fenderson et al 1987
SSEA4	IgG	Mouse	Human EC, ES cells.	↓ differentiation	Kannagi et al 1983 Fenderson et al 1987
A2B5	IgM	Mouse		↑ differentiation	Fenderson et al 1987
ME311	IgG	Mouse		↑ differentiation	Fenderson et al 1987
VIN-IS-56	IgM	Mouse		↑ differentiation	Andrews et al 1990
VIN-IS-53	IgG	Mouse		↑ differentiation	Andrews et al 1990

Table 8: Probes used to assess mRNA markers of differentiation

Gene	Cell Type
Synaptophysin	Neuron
NeuroD1	Neuron
MyoD1	Muscle
Collagens	Cartilage
Alpha-actin	Skeletal muscle
Smooth-muscle actin	Smooth muscle

Table 9: Protein markers of differentiation, detected by Western Blot and/or immunofluorescence.

The following antibodies were detected by the appropriate commercially available antibodies

Cell Type	Antigen
Neurons	Neurofilaments
Glial cells	GFAP
Epithelial cells	Cytokeratins
Mesenchymal cells	Vimentin
Muscle	Desmin
Muscle	Tissue specific actins
Connective tissue cells	Collagens

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Table 1

Notch receptor processing factors:

DEFINITION AL527440 LTI NFL003 NBC3 Homo sapiens Nrarp 5 Prime,partial mRNA sequence.

ACCESSION AL527440 SEQ ID NO: 1

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DEFINITION Homo sapiens E1A binding protein p300 (EP300), mRNA.

ACCESSION NM_001429 SEQ ID NO: 2

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DEFINITION Homo sapiens presenilin-associated protein mRNA, complete cds.

ACCESSION AF189289 SEQ ID NO: 3

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DEFINITION Homo sapiens presenilin 1 (PSEN1) mRNA, complete cds.

ACCESSION AF416717 SEQ ID NO: 4

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DEFINITION Homo sapiens presenilin 2 (Alzheimer disease 4) (PSEN2), transcript variant 1, mRNA.

ACCESSION NM_000447 SEQ ID NO: 5

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DEFINITION Homo sapiens F-box protein SEL10 (SEL10) mRNA, complete cds.

ACCESSION AY008274 SEQ ID NO: 6

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Target genes (transcription factors):

DEFINITION Homo sapiens HES-related repressor protein 1 HERP1 mRNA, complete SEQ ID NO: 7

cds.

ACCESSION AF232238

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DEFINITION Homo sapiens HES-related repressor protein 2 HERP2 mRNA, complete

cds.

ACCESSION AF232239 SEQ ID NO: 8

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DEFINITION Homo sapiens hairy (Drosophila)-homolog (HRY), mRNA.

ACCESSION NM_005524 SEQ ID NO: 9

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DEFINITION Homo sapiens hairy and enhancer of split (Drosophila) homolog 2 (HES2), mRNA. SEQ ID NO: 10

GenBank Acc: BG470458

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DEFINITION Homo sapiens bHLH factor Hes4 (LOC57801), mRNA.

ACCESSION NM_021170 SEQ ID NO: 11

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HESR1, SEQ ID NO:12

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DEFINITION Homo sapiens hairy/enhancer of split 6 (HES6) mRNA, complete cds.

ACCESSION AF260237 SEQ ID NO: 22

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DEFINITION Homo sapiens bHLH factor Hes7 (HES7), mRNA.

ACCESSION NM_032580 SEQ ID NO: 23

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Wnt pathway

Wnt ligand processing factors:

DEFINITION Homo sapiens low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor) (LRP1), mRNA.

ACCESSION XM_017228 SEQ ID NO:24

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DEFINITION Homo sapiens low density lipoprotein-related protein 2 (LRP2), mRNA.

ACCESSION XM_002645 SEQ ID NO:25

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DEFINITION Homo sapiens low density lipoprotein receptor-related protein 3

(LRP3), mRNA.
ACCESSION XM_036573 SEQ ID NO:26
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DEFINITION Homo sapiens low density lipoprotein receptor-related protein 4
(LRP4), mRNA.
ACCESSION XM_035037 SEQ ID NO:27
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DEFINITION Homo sapiens low density lipoprotein receptor-related protein 5
(LRP5), mRNA.

ACCESSION NM_002335 SEQ ID NO:28

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DEFINITION Homo sapiens low density lipoprotein receptor-related protein 6 (LRP6), Mrna.

ACCESSION NM_002336 SEQ ID NO:29

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DEFINITION Homo sapiens low density lipoprotein receptor-related protein 8, apolipoprotein e receptor (LRP8), mRNA.

ACCESSION XM_016537 SEQ ID NO:30

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DEFINITION Homo sapiens porcupine (MG61), mRNA.

ACCESSION NM_022825 SEQ ID NO:31
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Extracellular Wnt antagonists:
DEFINITION Homo sapiens dickkopf (Xenopus laevis) homolog 1 (DKK1), mRNA.
ACCESSION XM_048687 SEQ ID NO:32
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DEFINITION Homo sapiens dickkopf (Xenopus laevis) homolog 2 (DKK2), mRNA.
ACCESSION XM_052645 SEQ ID NO:33
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DEFINITION Homo sapiens dickkopf (Xenopus laevis) homolog 3 (DKK3), mRNA.

ACCESSION XM_006030 SEQ ID NO:34

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DEFINITION Homo sapiens dickkopf (Xenopus laevis) homolog 4 (DKK4), mRNA.

ACCESSION XM_032444 SEQ ID NO:35

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DEFINITION Human Frizzled related protein Frzb precursor (fzrb) mRNA, complete cds
ACCESSION U24163 SEQ ID NO:36
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DEFINITION Homo sapiens secreted apoptosis related protein 1 (SARP1) mRNA, partial cds.
ACCESSION AF017986 SEQ ID NO:37
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DEFINITION Homo sapiens adenomatosis polyposis coli (APC), mRNA.
ACCESSION XM_043933 SEQ ID NO:38
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DEFINITION Homo sapiens axin (AXIN1), mRNA.

ACCESSION XM_027520 SEQ ID NO:39

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DEFINITION Homo sapiens AXIN2 (AXIN2) mRNA, complete cds.

ACCESSION AF205888 SEQ ID NO:40

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ctgtcttggctgtgagcaactgcgacaaaacattttgaaggaaaa

DEFINITION Homo sapiens frequently rearranged in advanced T-cell lymphomas (FRAT1), mRNA.

ACCESSION XM_050913 SEQ ID NO:41

Attccggctcccgcggctgcaggcgcgcggctagagtgcctggcgggctccggcttccgcgtccgccccggccccggt
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DEFINITION Homo sapiens glycogen synthase kinase 3 beta (GSK3B), mRNA.

ACCESSION XM_010970 SEQ ID NO:42

Gaaggaaggaaaaggtgattcgcgaagagagtgatcatgtcagggcggcccagaaccacctcctttgcggagagctgc
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DEFINITION Homo sapiens beta-catenin-interacting protein ICAT (ICAT), mRNA.

ACCESSION NM_020248 SEQ ID NO:43

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DEFINITION Homo sapiens Dvl-binding protein IDAX mRNA, complete cds.

ACCESSION AF272159 SEQ ID NO:44

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aagggcaaactaatgtcttgttttaagaaactgcttagtccaccactgaagaaaatatccagaaattattttcattttatgtatagg
gatttcttcaaaaaaaaaaaaaaa

DEFINITION Homo sapiens orphan nuclear receptor (PAR1) mRNA, complete cds.

ACCESSION AF084645 SEQ ID NO:45

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DEFINITION Homo sapiens transforming growth factor beta-activated kinase-binding protein 1 (TAB1), mRNA.

ACCESSION XM_010000 SEQ ID NO:46

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Nuclear acting components:

DEFINITION H.sapiens mRNA for beta-catenin.

ACCESSION X87838 SEQ ID NO:47

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DEFINITION Homo sapiens b-TRCP variant E3RS-IkappaB mRNA, partial cds.
ACCESSION AF101784 SEQ ID NO:48
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DEFINITION Homo sapiens C-terminal binding protein 1 (CTBP1), mRNA.

ACCESSION NM_001328 SEQ ID NO:50

Cgcgcagcgcgccggagtggtcggggcccgcggccgctcgcgcctctcgatgggcagctcgcacttgctcaacaagg
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DEFINITION Homo sapiens HMG-box containing protein 1 (HBP1), mRNA.

ACCESSION XM_027193 SEQ ID NO:51

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aaatggaaaaa

DEFINITION Homo sapiens lymphoid enhancer factor-1 (LEF1) mRNA, complete cds.

ACCESSION AF288571 SEQ ID NO:52

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DEFINITION Homo sapiens nemo-like kinase mRNA, complete cds.

ACCESSION AF197898 SEQ ID NO:53

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acaaatttaaaaaaaaaa

DEFINITION Homo sapiens Pontin52 mRNA, complete cds.

ACCESSION AF099084 SEQ ID NO:54

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DEFINITION Homo sapiens Reptin52 mRNA, complete cds.

ACCESSION AF124607 SEQ ID NO:55

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Wnt target genes:
DEFINITION Homo sapiens achaete-scute complex (Drosophila) homolog-like 1 (ASCL1), mRNA.
ACCESSION NM_004316 SEQ ID NO:56
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DEFINITION Homo sapiens achaete-scute complex-like 2 (Drosophila) (ASCL2), mRNA.
ACCESSION NM_005170 SEQ ID NO:57
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DEFINITION Homo sapiens fibroblast growth factor 5 (FGF5), mRNA.
ACCESSION XM_054589 SEQ ID NO:58
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DEFINITION Homo sapiens muscle segment homeobox 1 (MSX1) gene, complete cds.

ACCESSION AF426432 SEQ ID NO:59

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DEFINITION Homo sapiens neurogenin 1 (NEUROG1), mRNA.

ACCESSION NM_006161 SEQ ID NO:60

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DEFINITION Homo sapiens neurogenin 2 gene, partial cds.
ACCESSION AF303002 SEQ ID NO:61
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DEFINITION Homo sapiens neurogenin 3 (NEUROG3), mRNA.
ACCESSION XM_005744 SEQ ID NO:62
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ACCESSION NM_000314 SEQ ID NO:63
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aaccttctgcaacatcttaagatccacaaatgaagggatataaaaataatgtcataggtaagaaacacagcaacaatgactta
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ttgtaaaaaaaaaa

Table 2 Polycomb Group.
EED
SEQ ID NO:64
>gi|14523051|ref|NM_003797.1| Homo sapiens embryonic ectoderm development (EED), Mrna
GAGGGAAGTGTCGACTGCGCCGGCGGGAACAGACATGCCTGCGGCCAAGAAGCAGAAGCTGAGCAGTGAC
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TGATGAAAACTTTTACACTTGTGCATGGACCTATGATAGCAATACGAGCCATCCTCTGCTGGCTGTAGCT
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YY1

SEQ ID NO:65

>gi|6042207|ref|NM_003403.2| Homo sapiens YY1 transcription factor (YY1), mRNA

AGGGCGAACGGGCGAGTGGCAGCGAGGCGGGGCGGGCTGAGGCCAGCGCGGAAGTCTCGCGAGGCCGGGC
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GCGGAGGCGCAGGAGGAGGAAGGGGGAAGCGGAGCGCGGCCCGGACGGGAGGAGGCGCGGCCAGGGCGGG
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GTATTTTCCTGTAAAAAAAAAAAAAGTTATATAGGTTTTGTTTGCTATCTTAATTTTGGTTGTATTCTTT
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CTGATGTTCAGTGTAATTTCTTTGCCTGTTCAGTTACAGAAAGTGGTGCTCAGTTGTAGAATGTATTGTA
CCTTTTAACACCTGATGTGTACATCCCATGTAACAGAAAGGGCAACAATAAAATAGCAATCCTAAAG

CBX1

SEQ ID NO:66

>gi|773207|emb|X77824.1|HSM3 H.sapiens chromobox 2 gene

CTGCCAGAGATGAGTGCAGGTGAGGAGAGTAGCAGCTCGGACTCCGACCCCGACTCCGCCTCGCCGCCCA
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GCACGTATTTGTCACCGACGTCACTGCCAACCTCATCACCGTCACAGTGAAGGAGTCTCCCACCAGCGTC

GGCTTCTTCAACCTGAGGCATTACTGAAGCCCCGGCGCCACCAGCTGCGCGTCTTACTCCCCTTCCCTGC
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AGTGGG

CBX6

SEQ ID NO:67

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Ccgagcccgaggctggggactggcgccccgagatgtcaccctgctccaatgtggtcgtca
Ccgatgtcaccagcaacctcctgacggtcacaatcaaggaattctgcaaccctgaggatt
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Gcaagtga

HPC2 (CBX4)

SEQ ID NO:68

>gi|4503032|ref|NM_001880.1| Homo sapiens activating transcription factor 2 (ATF2), mRNA
GAATTCTGTGATAAGTTATTCAACTTATGAAATTCAAGTTACATGTGAATTCTGCCAGGCAATACAAGGA
CCTGTGGAATATGAGTGATGACAAACCCTTTCTATGTACTGCGCCTGGATGTGGCCAGCGTTTTACCAAC
GAGGATCATTTGGCTGTCCATAAACATAAACATGAGATGACACTGAAATTTGGTCCAGCACGTAATGACA
GTGTCATTGTGGCTGATCAGACCCCAACACCAACAAGATTCTTGAAAAACTGTGAAGAAGTGGGTTTGTT
TAATGAGTTGGCGAGTCCATTTGAGAATGAATTCAAGAAAGCTTCAGAAGATGACATTAAAAAAATGCCT
CTAGATTTATCCCCTCTTGCAACACCTATCATAAGAAGCAAAATTGAGGAGCCTTCTGTTGTAGAAACAA
CTCACCAGGATAGTCCTTTACCTCACCCAGAGTCTACTACCAGTGATGAGAAGGAAGTACCATTGGCACA
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TCTGACTCAAGTGTAATTATTCAGCAGGCAGTACCTTCACCAACCTCAAGTACTGTAATCACCCAGGCAC
CATCCTCTAACAGGCCAATTGTCCCTGTACCAGGCCCATTTCCTCTTCTGTTACATCTTCCTAGTGGACA
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GTTCGACCAGTCACCATGGTGCCTAGTGTTCCAGGAATCCCAGGTCCTTCCTCTCCCCAACCAGTACAGT
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ACATCCAATGGAGTCAGTTCAACCTCCAAGGCAGAAGCTGTAGCCACTTCAGTCCTCACCCAGATGGCGG
ACCAGAGTACAGAGCCTGCTCTTTCACAGATCGTTATGGCTCCTTCCTCCCAGTCACAGCCCTCAGGAAG
TTGATTAAAAACCTGCAGTACAACAGTTTAGATACTCATTAGTGACTTCAAAGGGAAATCAAGGAAAGAC
CAGTTTCCATTTATGCGAAATCTGTGGTTGTAAATTT

HPC3

SEQ ID NO:69

>gi|10190681|ref|NM_020649.1| Homo sapiens chromobox homolog 8 (Pc class homolog, Drosophila) (CBX8), mRNA

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TAGGGGGGAAGCGGCGAGTCAACATGGAGCTTTCAGCGGTGGGGGAGCGGGTGTTCGCGGCCGAAGCCCT
CCTGAAGCGGCGCATACGGAAAGGACGCATGGAATACCTCGTGAAATGGAAGGGATGGTCGCAGAAGTAC
AGCACATGGGAACCGGAGGAAAACATCCTGGATGCTCGCTTGCTCGCAGCCTTTGAGGAAAGGGAAAGAG
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CGGGGTACCAGCAGAGTGGATGACAAGCCCAGCTCACCGGGGGACAGCTCGAAGAAGCGAGGCCCCAAGC
CCCGGAAGGAGCTCCCGGACCCCTCACAGAGGCCCTTAGGCGAACCCAGCGCCGGCCTCGGAGAGTACCT
CAAGGGCAGGAAGCTGGACGACACCCCTTCCGGGGCAGGAAAGTTTCCAGCCGGCCACAGTGTGATCCAG
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GCAAACTGGCTGTGGACACCTTCCCGGCCAGGGTGATAAAGCACAGGGCTGCCTTCCTGGAGGCCAAAGG
CCAGGGTGCCCTAGATCCCAATGGCACCCGGGTCCGACATGGCTCAGGCCCCCCCAGCTCTGGGGGGGGC
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TTCATCCCTCCTTCCTCCCCCTCAGTTTTTGTTGGAAAGATTATCTCTAGAGTTATATTTTCTATTAGAT
GTAAATATGTTATTTAAGAAAAATATCTAAATATATATATTTCAACTCGAAAAAAAAAAAAAAAAACTCG
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PHF1

SEQ ID NO:70

>gi|13435396|ref|NM_024165.1| Homo sapiens PHD finger protein 1 (PHF1), transcript variant 2, mRNA

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GGCCTGGGGACCCCCCGGCCTCCGCCTGCACGCCCCCCCACGCCCGGACGTGCCCTCTCCGCGCGGGGGA
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CGCAGTTTCTGGTTCTATGGAAAGACATTAGCCCTGCTGCCCTCCCTGGAGAGGAACTCCTCTGTTGTGT
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CCTGCCCAGCAGCCCCATCCGGATGTTTGCTTCCTTCCACCCTTCTGCCAGCACCGCAGGGACCTCTGGG
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CTACTGCCCAGGCTGGAATCCAAGAGTGGGGAGTGGGGAAGAGGCCCTCTTCTCTACCCTCCTTCATGAT
TCCTGACCCCTCCCATCCTTCCCATTTCCTTTGATGTTATTTTGTTACAGCTTTTTAAATATTTTTTAAA
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PHF2

SEQ ID NO:71

>gi|4885546|ref|NM_005392.1| Homo sapiens PHD finger protein 2 (PHF2), mRNA
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CCCTGCCCCAACATTCTACGTGAGTGACGTCGAGAACTACGTGGGGCCGGAACGGAGTGTGGATGTGACA
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CAATCAA

HPH1

SEQ ID NO:72

>gi|11038623|ref|NM_004426.1| Homo sapiens early development regulator 1 (polyhomeotic 1 homolog) (EDR1), mRNA

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AGCCGGCGCCCAGCCCAGCCTGGCGACTGGGGACCCCGGCACATGAGGTGGACGCCCCGGGGAAGACTTG
GGTGCACAGCCAGGCGAGAAGGTCTTGAGTCAGACAGAGCACCAGCCTTGGGGACCCTGGACCACTATCA
TGGAGACTGAGAGCGAGCAGAACTCCAATTCCACCAATGGGAGTTCTAGCTCAGGGGGCAGCTCTCGGCC
CCAGATAGCTCAAATGTCACTTTATGAACGACAAGCAGTGCAGGCTCTGCAAGCACTGCAGCGGCAGCCC
AATGCAGCTCAGTATTTCCACCAGTTCATGCTCCAGCAGCAGCTCAGTAATGCCCAGCTGCATAGCCTGG
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GCAGACTACCACCACCCAGGCCTCGATCAATCTGGCCACCACATCGGCCGCCCAGCTCATCAGCCGATCC
CAGAGTGTGAGCTCTCCCAGTGCTACCACCTTGACCCAATCTGTGCTACTGGGGAACACCACCTCCCCAC
CCCTCAACCAGTCTCAGGCCCAGATGTATCTACGGCCACAGCTGGGAAACCTATTGCAGGTAAACCGAAC
CCCTGGGTCGAATGTGCCTCTAGCCTCCCAACTCATCCTGATGCCTAATGGGGCGGTGGCTGCAGTCCAG
CAGGAGGTGCCATCTGCTCAGTCTCCTGGAGTTCATGCAGATGCAGATCAGGTTCAGAACTTGGCAGTAA
GGAATCAACAGGCCTCAGCTCAAGGACCTCAGATGCAAGGCTCCACTCAGAAGGCCATTCCTCCAGGAGC
CTCCCCTGTCTCTAGCCTCTCCCAGGCCTCTAGCCAGGCCCTAGCGGTGGCACAGGCTTCCTCTGGGGCC
ACAAACCAGTCCCTCAACCTTAGTCAAGCTGGTGGAGGCAGTGGGAATAGCATCCCAGGGTCCATGGGTC
CAGGTGGAGGTGGGCAGGCACATGGTGGTTTGGGTCAGTTGCCTTCCTCAGGAATGGGTGGTGGGAGCTG
TCCCAGGAAGGGTACAGGAGTGGTGCAGCCCTTGCCTGCAGCCCAAACAGTAACTGTGAGCCAGGGCAGC
CAGACAGAGGCAGAAAGTGCAGCAGCCAAGAAGGCAGAAGCAGATGGGAGTGGCCAGCAGAATGTGGGCA
TGAACCTGACACGGACAGCCACACCTGCGCCCAGCCAGACACTTATTAGCTCAGCCACCTACACACAGAT
CCAGCCCCATTCACTGATTCAGCAACAGCAACAGATCCACCTCCAGCAGAAACAGGTGGTGATCCAGCAG
CAGATTGCCATCCACCACCAGCAGCAGTTCCAGCACCGGCAGTCCCAGCTCCTTCACACAGCTACACACC
TCCAGTTGGCGCAGCAGCAGCAGCAGCAACAACAGCAACAGCAGCAGCCGCAAGCCACCAC
CCTCACTGCCCCTCAGCCACCACAGGTCCCACCTACTCAGCAGGTCCCACCTTCCCAGTCCCAGCAGCAA
GCCCAAACCCTGGTCGTTCAGCCCATGCTTCAGTCTTCACCCTTGTCTCTTCCACCTGATGCAGCCCCTA
AGCCACCAATTCCCATCCAATCCAAACCACCTGTAGCACCTATCAAGCCGCCTCAGTTAGGGGCCGCTAA
GATGTCAGCTGCCCAGCAACCACCACCCCATATCCCTGTGCAAGTTGTAGGCACTCGACAGCCAGGTACA
GCCCAGGCACAGGCTTTGGGGTTGGCACAGCTGGCAGCTGCTGTACCTACTTCCCGGGGGATGCCAGGTA
CAGTGCAGTCTGGTCAGGCCCTAAAGGCTTCCTCGCCACCTTCATCCCAGGCTCCTGGTGCACTGCAGGA
GTGCCCTCCCACATTGGCCCCGGGGATGACCCTTGCTCCTGTGCAGGGGACAGCACATGTGGTAAAGGGT
GGGGCTACCACCTCCACACCTGTTGTAGCCCAGGTCCCTGCTGCCTTCTATATGCAGTCTGTGCACTTGC
CGGGAAAACCCCAGACATTGGCTGTCAAACGCAAGGCTGACTCTGAGGAGGAGAGAGATGATGTCTCCAC
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AGTCTTGGAGAAAAAGCTGAATCAGTGGCTAATGTGAATGCTAATACTCCAAGCAGTGAACTAGTAGCCT
TGACCCCCGCCCCTTCAGTACCGCCTCCTACACTAGCCATGGTGTCTAGACAAATGGGTGACTCAAAACC
CCCACAGGCCATCGTGAAGCCCCAGATTCTCACCCACATCATTGAAGGCTTTGTTATCCAGGAAGGAGCA
GAACCTTTCCCCGGTGGTTGTTCTCAGTTACTGAAGGAGTCTGAGAAGCCACTACAGACTGGCCTTCCGA
CAGGCTTGACTGAGAATCAGTCAGGTGGCCCCTTTGGAGTGGACAGCCCATCTGCTGAGTTAGATAAGAA
GGCGAATCTCCTGAAGTGCGAGTACTGTGGGAAGTACGCCCCCGCAGAGCAGTTTCGTGGCTCTAAGAGG
TTCTGCTCCATGACCTGCGCTAAGAGGTACAATGTGAGCTGTAGCCATCAGTTCCGGCTGAAGAGGAAAA
AAATGAAAGAGTTTCAAGAAGCCAACTATGCTCGCGTTCGCAGGCGTGGACCCCGCCGCAGCTCCTCTGA
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TCCAGTTATGATGAAGCACTCTCTCCAACATCTCCTGGGCCTTTATCAGTAAGAGCTGGGCATGGAGAAC
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CTAAGGTGGCCCTCTTGCACAAACCAGCCTAAGGCAGACACTCTCCACTGTCCAGGTTATAACCTGGTAC
CAGCAGACTTTGCAGGGAAGAAAGAGTTGTTCCAATCATGTAACCTTCTGTAGGGGATTACTGAGACAGG
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TTATTTTTTAAAATCTTTACAGTTCTCACCATTTCACGTACCTTAATCCAATCTTTATAAAAGAGGCAGT
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GGGTTTTTCTAGCTTGTGTGACAGAAGTAGCAAAATCTGGTCCTCCCCCCTCCCAGTGTAGCTGTGGCTC
AGAGTTTTTTCTTTTTGTTGTCACTTACTCCCTTGTGATTGAATTTTTTCTCCTGCATCCATGGCAGGAT
CCCCAGCCAGTATAGAGACTTGGTTGGCATCTTCTGCTGCAGGGACTAAAAGTATTTGACTGGGGCACAT
GTGGCTGTTGTCATTCTTTCTGCATCCCACTGTTCCCCTCCAATTTATGTTATTTTCTACCCTGTTTTTC
AGTTCCATCTCTGCTCTGTCCTATAGCTTTATAAAACCAGAGTGTGTGGGGCTGAGGTCAGGAGTATAAG

TACCTGCCTTAGGCACTATTCCTTATATAACAAAAATATTAAATATTTTTTTCCTCAGTAAAAGGATG

HPH2

SEQ ID NO:73

>gi|4758241|ref|NM_004427.1| Homo sapiens early development regulator 2 (polyhomeotic 2 homolog) (EDR2), mRNA

GGCGCCGCATGTGTCTCCGCGGCGGCTGCAGCCCTCGAGCGCCCGCCGCCGCGCCCCAACCCCGGCCGCC
GCCCGCCCTCCCGCCCCGGCCTCGCGCCCCCGTCCCGGCCTCGCGCCCCGGCCGCCCTTTGTTGACGCCG
GCCAGGCCGTGCGGTCGGATGCGCCGCGGCAGCCCCGGGCCCCGGCTCGGAGGCTCCCGGGGCGAGAGGA
GGCGGCCCGCCGGCCGGGACCCCGCGCGAGTCGGCCCCGGCCAGGGGCTGCGTAGGCCCGCCCGGCCAGG
CCCAGCCGCCTGGACAGAGACAGGGCAGGGCATTGTTCATGCACTGACCGACCTCAGCATCCCCGGCATG
ACCTCAGGGAACGGAAACTCTGCCTCCAGCATCGCCGGCACTGCCCCCCAGAATGGTGAGAATAAACCAC
CACAGGCCATTGTGAAACCCCAAATCCTGACGCATGTTATCGAAGGGTTTGTGATCCAGGAGGGGGCGGA
CGTTTCCCGGTGGGACGCTCGTCTGCTGGTGGGGAATCTCAAGAAGAAGTATGCACAGGGGTTCCTGCCT
GAGAAACTTCCACAGCAGGATCACACCACCACCACTGACTCGGAGATGGAGGAGCCCTATCTGCAAGAAT
CCAAAGAGGAGGGTGCTCCCCTCAAACTCAAGTGTGAGCTCTGTGGCCGGGTGGACTTTGCCTATAAGTT
CAAGCGTTCCAAGCGCTTCTGTTCCATGGCTTGTGCAAAGAGGTACAACGTGGGATGCACCAAACGGGTG
GGACTTTTCCACTCAGACCGGAGCAAGCTGCAGAAGGCAGGAGCTGCGACCCACAACCGCCGTCGGCCAG
CAAAGCCAGTCTGCCACCACTTACCAAGGATACCAAGAAGCAGCCAACAGGCACTGTGCCCCTTTCGGTT
ACTGCTGCTTTGCGTAACACACAGCCAGGAAGACTCCAGCCGTTGCTCAGATAACTCAAGCTATGAGGAA
CCCTTGTCAGCCATCTCAGCCAGCTCATCTACTTCCGCCGGCGACAAGGCCAGCGGGACCTGGAGCTCCC
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AGAAGACGTCTACGAATTCATCCGCTCTCTGCCAGGCTGCCAGGAGATAGCAGAGGAATTCCGTGCCCAG
GAAATCGACGGGCAAGCCCTGCTGCTGCTCAAGGAGGACCACCTGATGAGCGTTATGAACATCAAGCTGG
GGCCCGCCCTGAAGATCTACGCCCGCATCAGCATGCTCAAGGACTCCTAGGGCTGGTGGCACCAGGATTC
TGGCCCAGGGCGCCTCCTCCCGACTGAGCAGAGCCAGACAGACATTCCTGAGGGGCCCAGAAATGGCGGC
GTTGGAGGGCAGGGGCTCTCCCTAGGGGCATAGCTGGTGAGGAGGTCTGGGCACCTCCTCCATGGCTCTC
AGGGGCCTTTCATTTCTGTGGGAGGGGCAGAGAGGTAGGTGGCACAGAAGATGGGGCTTTATGCTTGTAA
ATATTGATAGCACTGGCTTCCTCCAAAGTCCCAATACTCTAGCCCCGCTCTCTTCCCCTCTTTCTGTCCC
CCATTTTCCAGGGGGTATATGGTCAGGGCTCCCCAACCTGAGTTGGTTACTTCAAGGGCAGCCAGCAGGC
CTGGATGGAGGCCTAGAAAGCCCTTGCCTTCCTTCCTCCCACTTCTTTCTCCAGGCCTGGTTAACTCTTC
CGTTGTCAGCTTCTCCCCCTTCAGCCTGTTTCTGCAGCAGCCAGGGTTCTCCCCCCTACACCCTCTGCAG
GTGGAGAGAGAGAAGCTGGGCCCAGCCGCGGTGCCTGCTGGCCAAGACGCCTTAACGCTGTGTGTATGAC
TGTGTGACTGTGTGGGAGCCTGGACTGACAGATAGGCCAAGGGCTACTCTCTGGCATCTCCAGGTGTTTT
GTAGCAAACAGCCACTTAGTGCTTTGTCCTGGACTCCACTCAGCCTCAGGATGGGGAATAGCCAAGAATG
GCAGCCTCAGCGCAGAGGCAAGGTCAGAAAGAGACGGCGCTTCAGAGTTTCCTTTCCAGACACCCCTCCC
CGCACTGTGAAGTTCCCCTGACCGCCCTCCTGGTTCACAAAGAGCATTAAGAAAGCTGCGGTGGTCTGAG
CAACATAGCCCAGACGTGGAGCCTCCTGGCCTGCCTGCCCGCCCACCCTGGGAGTCCAGTGGTGAGGCTC
AGAGAACTTCTAAGGGGAAAGAACAGCTGGAGTTTCTGTTGATGTGAAGAAGGCAGCTCTTGGCCTCCCA
CTCCCACACTTCTTTGCCTATAAATCTTCCTAGCAGCAATTTGAGCTACCTGAGGAGGAGGCAGGGCAGA
AGGGCAAGGGCCTGCCTCTGACCTGCCGTGTCCTTTGCAGGAAGGAGGTAGGCACCTTTCTGAGCTTATT
CTATTCCCCACCCACACCCCCAGGCAGGGTTGGAAATGAAGGACTTTTTTAACCTTTGTTTTGTTTTTTA
AAAATAAATCTGTAAAATCTGAAAAAAAAAAAAAA

SSX1

SEQ ID NO:74

>gi|5032120|ref|NM_005635.1| Homo sapiens synovial sarcoma, X breakpoint 1 (SSX1), mRNA

CACTTTGTCACCAACTGCTGCCAACTCGCCACCACTGCTGCCGCAATCGCAACCACTGCTTTGTCTCTGA
AGTGAGACTGCTCCTGGTGCCATGAACGGAGACGACACCTTTGCAAAGAGACCCAGGGATGATGCTAAAG
CATCAGAGAAGAGAAGCAAGGCCTTTGATGATATTGCCACATACTTCTCTAAGAAAGAGTGGAAAAAGAT
GAAATACTCGGAGAAAATCAGCTATGTGTATATGAAGAGAAACTATAAGGCCATGACTAAACTAGGTTTC
AAAGTCACCCTCCCACCTTTCATGTGTAATAAACAGGCCACAGACTTCCAGGGGAATGATTTTGATAATG
ACCATAACCGCAGGATTCAGGTTGAACATCCTCAGATGACTTTCGGCAGGCTCCACAGAATCATCCCGAA
GATCATGCCCAAGAAGCCAGCAGAGGACGAAAATGATTCGAAGGGAGTGTCAGAAGCATCTGGCCCACAA
AACGATGGGAAACAACTGCACCCCCCAGGAAAAGCAAATATTTCTGAGAAGATTAATAAGAGATCTGGAC
CCAAAAGGGGGAAACATGCCTGGACCCACAGACTGCGTGAGAGAAAGCAGCTGGTGATTTATGAAGAGAT
CAGTGACCCTGAGGAAGATGACGAGTAACTCCCCTGGGGGATACGACACATGCCCTTGATGAGAAGCAGA
ACGTGGTGACCTTTCACGAACATGGGCATGGCTGCGGCTCCCTCGTCATCAGGTGCATAGCAAGTG

SSX2

SEQ ID NO:75

>gi|10337582|ref|NM_003147.1| Homo sapiens synovial sarcoma, X breakpoint 2 (SSX2), mRNA

CTCTCTTTCGATTCTTCCATACTCAGAGTACGCACGGTCTGATTTTCTCTTTGGATTCTTCCAAAATCAG
AGTCAGACTGCTCCCGGTGCCATGAACGGAGACGACGCCTTTGCAAGGAGACCCACGGTTGGTGCTCAAA
TACCAGAGAAGATCCAAAAGGCCTTCGATGATATTGCCAAATACTTCTCTAAGGAAGAGTGGGAAAAGAT
GAAAGCCTCGGAGAAAATCTTCTATGTGTATATGAAGAGAAAGTATGAGGCTATGACTAAACTAGGTTTC
AAGGCCACCCTCCCACCTTTCATGTGTAATAAACGGGCCGAAGACTTCCAGGGGAATGATTTGGATAATG
ACCCTAACCGTGGGAATCAGGTTGAACGTCCTCAGATGACTTTCGGCAGGCTCCAGGGAATCTCCCCGAA
GATCATGCCCAAGAAGCCAGCAGAGGAAGGAAATGATTCGGAGGAAGTGCCAGAAGCATCTGGCCCACAA
AATGATGGGAAAGAGCTGTGCCCCCCGGGAAAACCAACTACCTCTGAGAAGATTCACGAGAGATCTGGAC
CCAAAAGGGGGGAACATGCCTGGACCCACAGACTGCGTGAGAGAAAACAGCTGGTGATTTATGAAGAGAT
CAGCGACCCTGAGGAAGATGACGAGTAACTCCCCTCAGGGATACGACACATGCCCATGATGAGAAGCAGA
ACGTGGTGACCTTTCACGAACATGGGCATGGCTGCGGACCCCTCGTCATCAGGTGCATAGCAAGTG

Table 3 Enhancers of trithorax and polycomb.

EPC1

SEQ ID NO:76

>gi|13376809|ref|NM_025209.1| Homo sapiens enhancer of polycomb 1 (EPC1), mRNA

ATGAGTAAACTGTCGTTTCGGGCGCGGGCGCTAGACGCCTCGAAGCCGCTGCCGGTTTTCCGCTGTGAGG
ATCTGCCCGACCTGCACGAATACGCCTCGATAAACAGGGCCGTGCCGCAGATGCCCACCGGAATGGAGAA
GGAAGAGGAGTCGGAACATCATCTTCAGCGGGCTATTTCAGCACAGCAGGTGTATGGCGAGAAGAGGGAT
AATATGGTTATACCGGTCCCAGAGGCAGAAAGTAATATTGCTTACTATGAGTCTATATATCCTGGGGAAT
TTAAGATGCCAAAGCAGCTCATTCACATACAGCCTTTTAGTTTGGATGCTGAACAGCCTGATTATGATTT
GGATTCTGAAGATGAAGTATTTGTGAATAAACTGAAAAAGAAAATGGACATCTGCCCATTGCAATTTGAG
GAGATGATTGACCGCCTAGAAAAAGGCAGTGGTCAGCAGCCAGTCAGTCTGCAGGAAGCCAAACTACTGC
TAAAAGAAGATGATGAACTAATTAGAGAAGTTTATGAATATTGGATTAAAAAGAGAAAAAACTGTCGAGG
GCCATCTCTTATTCCATCAGTAAAACAAGAGAAGCGAGATGGTTCCAGCACAAATGATCCTTATGTGGCT
TTTAGAAGGCGTACTGAAAAAATGCAGACTCGAAAAAATCGCAAAAATGATGAAGCCTCTTACGAAAAAA
TGCTTAAGCTGCGACGAGATCTAAGTCGAGCTGTTACTATTCTAGAGATGATAAAAAGAAGAGAAAAAAG
TAAAAGAGAGCTATTGCACTTAACACTGGAAATTATGGAAAAGAGGTATAATTTGGGCGACTACAATGGA
GAGATCATGTCTGAGGTTATGGCACAGAGACAGCCAATGAAACCTACTTATGCCATCCCCATCATCCCTA
TTACTAATAGCAGTCAATTTAAACACCAGGAAGCAATGGATGTGAAGGAGTTCAAAGTTAATAAGCAAGA
TAAAGCCGATCTTATCCGACCGAAACGGAAATATGAAAAGAAGCCCAAAGTCTTACCATCGTCTGCCGCT
GCTACTCCCCAACAGACGAGTCCTGCTGCACTGCCAGTCTTCAATGCTAAAGATCTGAATCAGTATGACT
TTCCCAGCTCAGACGAAGAACCTCTCTCCCAGGTTTTGTCTGGCTCTTCGGAAGCTGAGGAAGACAATGA
TCCTGATGGTCCTTTTGCTTTCCGTAGGAAAGCAGGCTGTCAGTACTATGCTCCTCACTTAGACCAAACT
GGCAACTGGCCTTGGACTAGTCCTAAAGATGGAGGATTAGGGGATGTGCGATATAGATACTGCTTAACTA
CTCTCACCGTACCCCAAAGGTGTATTGGATTTGCACGAAGACGGGTTGGGCGCGGTGGAAGGGTCTTACT
GGACAGAGCTCATTCAGACTATGACAGTGTGTTTCACCATCTGGATTTGGAAATGCTTTCCTCACCACAA
CATTCTCCAGTCAATCAGTTTGCCAATACCTCAGAAACAAATACCTCGGACAAATCTTTCTCTAAAGACC
TCAGTCAGATACTAGTCAATATCAAATCATGTAGATGGCGGCATTTTAGGCCTCGGACACCATCCCTACA
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CCTGGGACCCAGACATGCAGTACCTCTACGCAAAGTAAAAGTAGCAGTGGTTCAGCACACTTTGCATTTA
CAGCCGAACAATACCAGCAACATCAACAGCAACTGGCACTCATGCAGAAACAGCAGCTTGCACAAATTCA
GCAACAGCAAGCAAATAGTAATTCCTCCACCAACACATCACAGAACCTTGCATCTAACCAGCAGAAAAGT
GGCTTTCGCCTGAATATACAGGGTTTAGAAAGAACACTACAGGGTTTTGTTTCTAAGACTTTGGATTCTG
CTAGTGCACAGTTTGCTGCTTCTGCTTTGGTGACATCAGAACAACTGATGGGATTCAAGATGAAGGATGA
TGTGGTGCTTGGAATCGGGGTGAATGGCGTCCTTCCAGCCTCAGGAGTATACAAGGGCTTACACCTCAGT
AGTACTACACCAACAGCACTTGTACATACAAGTCCATCAACGGCAGGTTCAGCTTTGTTACAGCCTTCAA
ATATTACACAGACTTCAAGTTCCCACAGTGCACTGAGTCATCAAGTAACTGCTGCCAATTCTGCAACAAC
TCAGGTTCTGATTGGGAACAACATTCGATTAACTGTACCTTCATCAGTTGCCACTGTAAACTCTATTGCC
CCAATAAATGCACGACATATACCTAGGACTTTAAGTGCTGTTCCATCATCTGCCTTAAAGCTGGCCGCTG
CAGCAAACTGTCAAGTTTCCAAGGTCCCATCTTCATCCTCTGTAGATTCAGTTCCAAGGGAAAATCATGA
ATCAGAAAAGCCAGCACTGAACAACATAGCAGACAACACAGTAGCGATGGAGGTGACGTAG

EZH1

SEQ ID NO:77

>gi|4503622|ref|NM_001991.1| Homo sapiens enhancer of zeste homolog 1 (Drosophila) (EZH1), mRNA

GAGGCTGGACACCTGTTCTGCTGTTGTGTCCTGCCATTCTCCTGAAGAACAGAGGCACACTGTAAAACCC
AACACTTCCCCTTGCATTCTATAAGATTACAGCAAGATGGAAATACCAAATCCCCCTACCTCCAAATGTA
TCACTTACTGGAAAAGAAAAGTGAAATCTGAATACATGCGACTTCGACAACTTAAACGGCTTCAGGCAAA
TATGGGTGCAAAGGCTTTGTATGTGGCAAATTTTGCAAAGGTTCAAGAAAAAACCCAGATCCTCAATGAA
GAATGGAAGAAGCTTCGTGTCCAACCTGTTCAGTCAATGAAGCCTGTGAGTGGACACCCTTTTCTCAAAA
AGTGTACCATAGAGAGCATTTTCCCGGGATTTGCAAGCCAACATATGTTAATGAGGTCACTGAACACAGT
TGCATTGGTTCCCATCATGTATTCCTGGTCCCCTCTCCAACAGAACTTTATGGTAGAAGATGAGACGGTT
TTGTGCAATATTCCCTACATGGGAGATGAAGTGAAAGAAGAAGATGAGACTTTTATTGAGGAGCTGATCA
ATAACTATGATGGGAAAGTCCATGGTGAAGAAGAGATGATCCCTGGATCCGTTCTGATTAGTGATGCTGT
TTTTCTGGAGTTGGTCGATGCCCTGAATCAGTACTCAGATGAGGAGGAGGAAGGGCACAATGACACCTCA
GATGGAAAGCAGGATGACAGCAAAGAAGATCTGCCAGTAACAAGAAAGAGAAAGCGACATGCTATTGAAG
GCAACAAAAAGAGTTCCAAGAAACAGTTCCCAAATGACATGATCTTCAGTGCAATTGCCTCAATGTTCCC
TGAGAATGGTGTCCCAGATGACATGAAGGAGAGGTATCGAGAACTAACAGAGATGTCAGACCCCAATGCA
CTTCCCCCTCAGTGCACACCCAACATCGATGGCCCCAATGCCAAGTCTGTGCAGCGGGAGCAATCTCTGC
ACTCCTTCCACACACTTTTTTGCCGGCGCTGCTTTAAATACGACTGCTTCCTTCACCCTTTTCATGCCAC
CCCTAATGTATATAAACGCAAGAATAAAGAAATCAAGATTGAACCAGAACCATGTGGCACAGACTGCTTC
CTTTTGCTGGAAGGAGCAAAGGAGTATGCCATGCTCCACAACCCCCGCTCCAAGTGCTCTGGTCGTCGCC
GGAGAAGGCACCACATAGTCAGTGCTTCCTGCTCCAATGCCTCAGCCTCTGCTGTGGCTGAGACTAAAGA
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TGGAATGGACTGGGGCTGAAGAATCTCTTTTTCGAGTCTTCCATGGCACCTACTTCAACAACTTCTGTTC
AATAGCCAGGCTTCTGGGGACCAAGACGTGCAAGCAGGTCTTTCAGTTTGCAGTCAAAGAATCACTTATC
CTGAAGCTGCCAACAGATGAGCTCATGTACCCCTCACAGAAGAAGAAAAGAAAGCACAGATTGTGGGCTG
CACACTGCAGGAAGATTCAGCTGAAGAAAGATAACTCTTCCACACAAGTGTACAACTACCAACCCTGCGA
CCACCCAGACCGCCCCTGTGACAGCACCTGCCCCTGCATCATGACTCAGAATTTCTGTGAGAAGTTCTGC
CAGTGCAACCCAGACTGTCAGAATCGTTTCCCTGGCTGTCGCTGTAAGACCCAGTGCAATACCAAGCAAT
GTCCTTGCTATCTGGCAGTGCGAGAATGTGACCCTGACCTGTGTCTCACCTGTGGGGCCTCAGAGCACTG
GGACTGCAAGGTGGTTTCCTGTAAAAACTGCAGCATCCAGCGTGGACTTAAGAAGCACCTGCTGCTGGCC
CCCTCTGATGTGGCCGGATGGGGCACCTTCATAAAGGAGTCTGTGCAGAAGAACGAATTCATTTCTGAAT
ACTGTGGTGAGCTCATCTCTCAGGATGAGGCTGATCGACGCGGAAAGGTCTATGACAAATACATGTCCAG
CTTCCTCTTCAACCTCAATAATGATTTTGTAGTGGATGCTACTCGGAAAGGAAACAAAATTCGATTTGCA
AATCATTCAGTGAATCCCAACTGTTATGCCAAAGTGGTCATGGTGAATGGAGACCATCGGATTGGGATCT
TTGCCAAGAGGGCAATTCAAGCTGGCGAAGAGCTCTTCTTTGATTACAGGTACAGCCAAGCTGATGCTCT
CAAGTACGTGGGGATCGAGAGGGAGACCGACGTCCTTTAGCCCTCCCAGGCCCCAACGGCAGCACTTATG
GTAGCGGCACTGTCTTGGCTTTCGTGCTCACACCACTGCTGCTCGAGTCTCCTGCACTGTGTCTCCCACA
CTGAGAAACCCCCCAACCCACTCCCCCTGTAGTGAGGCCTCTGCCATGTCCAGAGGGCACAAAACTGTCT
CAATGAGAGGGGAGACAGAGGCAGCTAGGGCTTGGTCTCCCAGGACAGAGAGTTACAGAAATGGGAGACT
GTTT

EZH2

SEQ ID NO:78

gi|4758323|ref|NM_004456.1| Homo sapiens enhancer of zeste homolog 2 (Drosophila) (EZH2), mRNA

GAATTCCGGGCGACGCGCGGGAACAACGCGAGTCGGCGCGCGGGACGAAGAATAATCATGGGCCAGACTG
GGAAGAAATCTGAGAAGGGACCAGTTTGTTGGCGGAAGCGTGTAAAATCAGAGTACATGCGACTGAGACA
GCTCAAGAGGTTCAGACGAGCTGATGAAGTAAAGAGTATGTTTAGTTCCAATCGTCAGAAAATTTTGGAA
AGAACGGAAATCTTAAACCAAGAATGGAAACAGCGAAGGATACAGCCTGTGCACATCCTGACTTCTGTGA
GCTCATTGCGCGGGACTAGGGAGTGTTCGGTGACCAGTGACTTGGATTTTCCAACACAAGTCATCCCATT
AAAGACTCTGAATGCAGTTGCTTCAGTACCCATAATGTATTCTTGGTCTCCCCTACAGCAGAATTTTATG
GTGGAAGATGAAACTGTTTTACATAACATTCCTTATATGGGAGATGAAGTTTTAGATCAGGATGGTACTT
TCATTGAAGAACTAATAAAAAATTATGATGGGAAAGTACACGGGGATAGAGAATGTGGGTTTATAAATGA
TGAAATTTTTGTGGAGTTGGTGAATGCCCTTGGTCAATATAATGATGATGACGATGATGATGATGGAGAC
GATCCTGAAGAAAGAGAAGAAAAAGCAGAAAGATCTGGAGGATCACCGAGATGATAAAGAAAGCCGCCCAC

CTCGGAAATTTCCTTCTGATAAAATTTTGGAGGCCATTTCCTCAATGTTTCCAGATAAGGGCACAGCAGA
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CCCAACATAGATGGACCAAATGCTAAATCTGTTCAGAGAGAGCAAAGCTTACACTCCTTTCATACGCTTT
TCTGTAGGCGATGTTTTAAATATGACTGCTTCCTACATCCTTTTCATGCAACACCCAACACTTATAAGCG
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AGACAGTGATAGGGAAGCAGGGACTGAAACGGGGGGAGAGAACAATGATAAAGAAGAAGAAGAGAAGAAA
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TGACAATTTCTGTGCCATTGCTAGGTTAATTGGGACCAAAACATGTAGACAGGTGTATGAGTTTAGAGTC
AAAGAATCTAGCATCATAGCTCCAGCTCCCGCTGAGGATGTGGATACTCCTCCAAGGAAAAAGAAGAGGA
AACACCGGTTGTGGGCTGCACACTGCAGAAAGATACAGCTGAAAAAGGACGGCTCCTCTAACCATGTTTA
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TTTTGTGAAAAGTTTTGTCAATGTAGTTCAGAGTGTCAAAACCGCTTTCCGGGATGCCGCTGCAAAGCAC
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AAGCATCTATTGCTGGCACCATCTGACGTGGCAGGCTGGGGGATTTTTATCAAAGATCCTGTGCAGAAAA
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TGATAAATACATGTGCAGCTTTCTGTTCAACTTGAACAATGATTTTGTGGTGGATGCAACCCGCAAGGGT
AACAAAATTCGTTTTGCAAATCATTCGGTAAATCCAAACTGCTATGCAAAAGTTATGATGGTTAACGGTG
ATCACAGGATAGGTATTTTTGCCAAGAGAGCCATCCAGACTGGCGAAGAGCTGTTTGTTGATTACAGATA
CAGCCAGGCTGATGCCCTGAAGTATGTCGGCATCGAAAGAGAAATGGAAATCCCTTGACATCTGCTACCT
CCTCCCCCTCCTCTGAAACAGCTGCCTTAGCTTCAGGAACCTCGAGTACTGTGGGCAATTTAGAAAAAGA
ACATGCAGTTTGAAATTCTGAATTTGCAAAGTACTGTAAGAATAATTTATAGTAATGAGTTTAAAAATCA
ACTTTTTATTGCCTTCTCACCAGCTGCAAAGTGTTTTGTACCAGTGAATTTTTGCAATAATGCAGTATGG
TACATTTTTCAACTTTGAATAAAGAATACTTGAACTTGAAAAAAAAAAAAAAAAAA

BMI1

SEQ ID NO:79

>gi|4885094|ref|NM_005180.1| Homo sapiens B lymphoma Mo-MLV insertion region (mouse) (BMI1), mRNA

GAGAGGCAGAGATCGGGGCGAGACAATGGGGATGTGGGCGCGGGAGCCCCGTTCCGGCTTAGCAGCACCT
CCCAGCCCCGCAGAATAAAACCGATCGCGCCCCCTCCGCGCGCGCCCTCCCCCGAGTGCGGAGCGGGAGG
AGGCGGCGGCGGCCGAGGAGGAGGAGGAGGAGGCCCCGGAGGAGGAGGCGTTGGAGGTCGAGGCGGAGGC
GGAGGAGGAGGAGGCCGAGGCGCCGGAGGAGGCCGAGGCGCCGGAGCAGGAGGAGGCCGGCCGGAGGCGG
CATGAGACGAGCGTGGCGGCCGCGGCTGCTCGGGGCCGCGCTGGTTGCCCATTGACAGCGGCGTCTGCAG
CTCGCTTCAAGATGGCCGCTTGGCTCGCATTCATTTTCTGCTGAACGACTTTTAACTTTCATTGTCTTTT
CCGCCCGCTTCGATCGCCTCGCGCCGGCTGCTCTTTCCGGGATTTTTTATCAAGCAGAAATGCATCGAAC
AACGAGAATCAAGATCACTGAGCTAAATCCCCACCTGATGTGTGTGCTTTGTGGAGGGTACTTCATTGAT
GCCACAACCATAATAGAATGTCTACATTCCTTCTGTAAAACTTGTATTGTTCGTTACCTGGAGACCAGCA
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TCTCCAAGATATTGTATACAAATTAGTTCCAGGGCTTTTCAAAAATGAAATGAAGAGAAGAAGGGATTTT
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GCAGCAATGACTGTGATGCACTTAAGAAAGTTTCTCAGAAGTAAAATGGACATACCTAATACTTTCCAGA
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TTGTAAGTGTTGTTTCTAATTATAGATGTAAAATGAAATTTCATTTGTAATTGGAAAAAATCCAATAAAA
AGGATATTCATTTAGAAAATAGCTAAGATCTTTAATAAAAATTTGATATGAAA

ZNF144(MEL18)

SEQ ID NO:80

>gi|6005963|ref|NM_007144.1| Homo sapiens zinc finger protein 144 (Mel-18) (ZNF144), mRNA

GAGAGCCCGAACAGGAAGAGGGTACAGCTTTGTGCAGGTCACATGCCCACTGCAGCCCTCCAGCCTCTGG
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ACGCCACCACTATCGTGGAGTGCCTGCATTCCTTCTGCAAAACCTGCATCGTGCGCTACCTGGAGACCAA
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TCTTGTGGAAAAGAAGATTCAGGCCCTGAGAGGTCTCAGCTCTTGGAGGAGGGCTAAGGCTTTAGCATTG
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TCACCTGGGGCCTAGAGTGGAAGTGGGGGTGGGTTAACCTCACACAAGCACAGATCCCAGACTTTGCCAG
AGGCAAACAGGGAATTCCGCCGATACTGACGGGCTCCAGGAGTCGTCGCCACACTCG

SCML1

SEQ ID NO:81

>gi|5803158|ref|NM_006746.1| Homo sapiens sex comb on midleg-like 1 (Drosophila) (SCML1), mRNA

ATCACTGGTCTCGCGTGCGCGTGACCAGGCCCGGTTTCCGGTGCCAGGACCTTTCCGAAGCGTCGAGTGG
CCTAACGGTCACAGCTGTCGCCCATCGGAGAGGCAGGACTACTGCGAGCAGTTTTACCGCGACCTCCGGA

GGCCGGCGTGACAGGCTCTGTCACTAAAATAGGAACCGAATATTGTATCTGACGCATCCTGTAATACTGA
AGAGCAACTGAAGACAGTTGATGATGTCCTTATTCATTGCCAGGTTATATATGATGCTCTGCAAAACCTG
GATAAGAAGATTGATGTGATTCGTAGAAAGGTTTCAAAAATCCAACGTTTCCATGCGAGATCCCTGTGGA
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GAAGAAAAATGAGGTTTACGAGACATTCTCCTACCCTGAAAGTTACAGCCCCACTTTACCAGTGTCAAGG
CGTGAGAATAATTCCCCGAGCAACCTTCCAAGGCCATCCTTTTGCATGGAAGAATACCAGCGAGCTGAGC
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TCAAGGGAAACATGTTCAACACTATTATTTTGTTATAAATTTATAACTTTGTTATTACATTGTGTAACAA
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TTAAAGAATAGCTTCATCAATGTTATTCCTTACATGTCATAAGATTCTTACTTAAACTTGGTCTTCTTTC
AAATTGTTTGTATGAAGATGCTGTACCCACTTGAACAGTCCTCAGGTGTTTACATAAATACTATGTTTTA
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SCML2

SEQ ID NO:82

>gi|5174668|ref|NM_006089.1| Homo sapiens sex comb on midleg-like 2 (Drosophila) (SCML2), mRNA
GAAGCTCGGTGCCGGCTCGCGCGATCGGTGGGACAGAATTTCGTTGTTTTCACCGACGAGACTGGAGGAA
ACAACACCAAATAGGGATACCATGGGACAAACAGTGAATGAAGATTCCATGGATGTCAAGAAGGAGAATC
AAGAGAAAACTCCTCAGTCAAGTACATCTTCTGTACAAAGGGATGATTTCCACTGGGAGGAGTATTTGAA
AGAGACTGGGTCTATAAGTGCTCCTTCAGAGTGCTTCCGTCAGTCTCAGATTCCACCTGTGAATGATTTC
AAAGTTGGTATGAAATTGGAAGCCCGTGACCCTCGCAATGCCACTTCAGTATGTATTGCTACGGTTATTG
GAATTACTGGGGCCAGGTTACGGTTACGACTGGATGGTAGTGACAACAGAAATGATTTTTGGAGGCTTGT
CGATTCCCCAGACATACAACCTGTTGGGACATGTGAAAAGGAAGGAGACTTACTTCAACCTCCACTAGGG
TACCAGATGAATACATCCTCCTGGCCGATGTTCCTCTTAAAGACACTAAATGGGTCTGAAATGGCATCTG
CCACATTATTTAAGAAGGAACCACCAAAGCCCCCACTAAATAATTTTAAAGTGGGGATGAAACTGGAAGC
TATTGACAAAAAGAACCCGTATCTCATCTGTCCTGCGACCATTGGAGATGTTAAAGGGGATGAAGTTCAT
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GGAGCAGTTCTGTTAAAAATATCACACCAAGGAAAAAAGGTCCAAACTCAGGAAAAAAGGAAAAACCTTT
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AAGCCAGATAATCGTGGAGGAGAAGTGATAACTGCCTCCTTTGATGGGGAAACTCATTCCATCCAGCTCC
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CCTTTTGAGTAGCCAGCCTTTTAGTTCTTCCAGGGGTCATACTCACAGCTCTGCAGAGCATGATAAAAAT
CAGTCAGCAAAAGAAGATGTAACAGAAAGGCAAAGCACCAAACGATCTCCTCAGCAAACTGTACCATATG
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TATTTGAATCCTGCCTGTAGAAATCCTATGTATATTCATACTTCAGTCTCCCAGGATTTTTCTCGAAGTG
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CCAAATGCAGAGGAAAAGTGAAGCTCCAAGTTATATAGCTGTACCTGATCCCAGTGTCCTGAAACAAGGC
TTCTCTAAGGACCCTTCAACCTGGTCTGTGGATGAAGTGATACAGTTTATGAAACATACAGATCCTCAGA
TATCAGGCCCCCTCGCCGACCTCTTCAGGCAACATGAAATTGATGGGAAGGCTCTGTTCCTACTCAAGAG
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CAGGAATGCACCACCACGCCCAGCTAATTTTGTAGAGATGGGGTTTCTCCATGTTGGTCAGGCTGGTCTC
AAACTCCTGACCTCAGGTGATCCGCCCACCTCGGCCTTGCAAAATGCTGGGATTACAGGCATGAGCCACC
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TGTTATTACTTTGTGTAACTTTGATGTAATGTTTATAAGCTATGAGAATCAGTTATAAAAGTATTAGCCA
TTTGTTGTAAATGCCAATAAAATATTCACCAGGGGCAAGAATGTACATTTTCTTTTTAGAAAACCAAATG
TACTTTAGACATGAATGCAACTATTTAAAGAATAGCTTCATTTATGTTATTCCTTACATGTCATAAGATT
CTTACTTAAACTTGGTCTTCTTTCAAGTTGTTTGTATGAAGATGCTGTACCCACTTGAACAGTCCTCAGG
TGTTTACATAAATACTATGTTTTACAGTTTTCATATTTTAAAATATTAATAAAGTTAATCGCAACGATTC

RING1

SEQ ID NO:83

>gi|11863157|ref|NM_002931.2| Homo sapiens ring finger protein 1 (RING1), mRNA

GGCTGCTGTTTCTAAAACCCCTTTCCCTCTAACCCACACCACCTTTCTACTCACTGATGCCTTCAGGAAG
CCATAATGGATGGCACAGAGATTGCTGTTTCCCCTCGGTCACTGCATTCAGAACTCATGTGCCCTATCTG
CCTGGACATGCTGAAGAATACGATGACCACCAAGGAGTGCCTCCACAGATTCTGCTCTGACTGCATTGTC
ACAGCCCTACGGAGCGGGAACAAGGAGTGTCCTACCTGCCGAAAGAAGCTGGTGTCCAAGCGATCCCTAC
GGCCAGACCCCAACTTTGATGCCCTGATCTCTAAGATCTATCCTAGCCGGGAGGAATACGAGGCCCATCA
AGACCGAGTGCTTATCCGCCTGAGCCGCCTGCACAACCAGCAGGCATTGAGCTCCAGCATTGAGGAGGGG
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CACCAAGGATCCAAAGTGACCCCACCAGGGGACAGCCAGAGGAAGGGGACCATGGGGTATCCCTGTGTCC
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GCCCCCATCTGCTGCCTTTTCTATTGCCCTGCAACGTCCCATCTATACGAGGTGTTGGAGAAGGTGAAGA
ACCCTCCCATTCACGCCCGCCTACCAACAACAAACGTGCTTTTTTCCTCTTTGAAAAAA

RYBP

SEQ ID NO:84

>gi|6912639|ref|NM_012234.1| Homo sapiens RING1 and YY1 binding protein (RYBP), mRNA

CCCGGACGGCGTTTCTCCTCCGAGCGGCGCCGGTTTCGGCTTGGGGGGGGCGGGGTACAGCCCATCCATG
ACCATGGGCGACAAGAAGAGCCCGACCAGGCCAAAAAGACAAGCGAAACCTGCCGCAGACGAAGGGTTTT
GGGATTGTAGCGTCTGCACCTTCAGAAACAGTGCTGAAGCCTTTAAATGCAGCATCTGCGATGTGAGGAA
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ACCCCACCACCCCCTAAAAAGGAGAAGAAGGAGAAAGTTGAAAAGCAGGACAAAGAGAAACCTGAGAAAG
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GAAAGATCCTCCTAGTGAAGCAAACAGCATACAGTCTGCAAATGCTACAACAAAGACCAGCGAAACAAAT
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CGTTCCTCCACGCCAAAGGGCGACATGTCAGCAGTCAATGATGAATCTTTCTGAAATTGCACATGGAATT
GTGAAAACTATGAATCAGGGTATGAAATTCAAAACCTCCACCTGCCCATGCTGCTTGCATCCCTGGAGAA
TCTTCTGTGGACATCGACCTCTTAGTGATGCTGCCAGGATAATTTCTGCTTGCCATGGGCATCTGGCCAC
CAAGGAATTTCGCACCCTGACGATTACTCTTGACACTTTTATGTATTCCATTGTTTTATATGATTTTCCT
AACAATCATTTATAATTGGATGTGCTCCTGAATCTACTTTTAATAAAAAAAAAAAA

MLL

SEQ ID NO: 85

>gi|5174568|ref|NM_005933.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila) (MLL), mRNA

ATGGCGCACAGCTGTCGGTGGCGCTTCCCCGCCCGACCCGGGACCACCGGGGGCGGCGGCGGCGGGGGGC
GCCGGGGCCTAGGGGGCGNCCCGCGGCAACGCGTCCCGGCCCTGCTGCTTCCCCCCGGGCCCCCGGTCGG
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CGTCATCGTCCTCAGCCTCTTCAGGGCCGGCCCTGCTCCGGGTGGGCCCGGGCTTCGACGCGGCGCTGCA
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GCGAAAATCTATTTTGCGAGAACCGACATTTAGGTGGACTTCTTTAAAGCATTCTAGGTCAGAGCCACAA
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MLLT1

SEQ ID NO:86

>gi|5174570|ref|NM_005934.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 1 (MLLT1), mRNA

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MLLT7

SEQ ID NO:87

>gi|5174578|ref|NM_005938.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 7 (MLLT7), mRNA

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MLLT6

SEQ ID NO:88

>gi|5174576|ref|NM_005937.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6 (MLLT6), mRNA

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MLLT4

SEQ ID NO:89

>gi|5174574|ref|NM_005936.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4 (MLLT4), mRNA

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MLLT3

SEQ ID NO:90

gi|4758719|ref|NM_004529.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3 (MLLT3), mRNA

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MLLT2

SEQ ID NO:91

>gi|5174572|ref|NM_005935.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 2 (MLLT2), mRNA

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MLLT10

SEQ ID NO:92

>gi|4757725|ref|NM_004641.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10 (MLLT10), mRNA

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MLL2

SEQ ID NO:93

>gi|4505196|ref|NM_003482.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia 2 (MLL2), mRNA

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Table 4 Histone Deacetylases

HDAC1

SEQ ID NO:94

>gi|13128859|ref|NM_004964.2| Homo sapiens histone deacetylase 1 (HDAC1), mRNA
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HDAC2

SEQ ID NO:95

>gi|4557640|ref|NM_001527.1| Homo sapiens histone deacetylase 2 (HDAC2), mRNA
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HDAC3

SEQ ID NO:96

>gi|13128861|ref|NM_003883.2| Homo sapiens histone deacetylase 3 (HDAC3), mRNA

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HDAC4

SEQ ID NO:97

>gi|13259519|ref|NM_006037.2| Homo sapiens histone deacetylase 4 (HDAC4),

mRNA
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HDAC5

SEQ ID NO:98

>gi|13259520|ref|NM_005474.2| Homo sapiens histone deacetylase 5 (HDAC5), mRNA

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HDAC6

SEQ ID NO:99

>gi|13128863|ref|NM_006044.2| Homo sapiens histone deacetylase 6 (HDAC6), mRNA
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HDAC7

SEQ ID NO:100

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>gi|13259523|ref|NM_016596.2| Homo sapiens histone deacetylase 7A (HDAC7A), transcript variant 2, mRNA SEQ ID NO:101

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CTCTCCAGCTCAGAGACCCCTGCCAGGACCCTGCCCTTCACCACAGGGCTGATCTATGACTCGGTCATGC
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TGGACAACGGGAAGCTGGCAGGGCTCCTGGCACAGCGGATGTTTGTGATGCTGCCCTGTGGTGGGGTTGG
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CAAACCTTCTACCAAGACCCCAGTGTGCTCTACATCTCCCTGCATCGCCATGACGACGGCAACTTCTTCC
CGGGGAGTGGGGCTGTGGATGAGGTAGGGGCTGGCAGCGGTGAGGGCTTCAATGTCAATGTGGCCTGGGC
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GCCCGAGAGTTCTCTCCAGACCTAGTCCTGGTGTCTGCTGGATTTGATGCTGCTGAGGGTCACCCGGCCC
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ATTTGCTTTAAAAAAAAAAAAAAAAA

>gi|7662279|ref|NM_014707.1| Homo sapiens histone deacetylase 9 (HDAC9-PENDING), transcript variant 3, mRNA SEQ ID NO:102

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CCAACTAATGGAAAAAATCATTCCGTGAGCCGCCATCCCAAGCTCTGGTACACGGCTGCCCACCACACAT
CATTGGATCAAAGCTCTCCACCCCTTAGTGGAACATCTCCATCCTACAAGTACACATTACCAGGAGCACA

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CCCCGTCACAGACCCCTGAACCGAACCCAGTCTGCACCTTTGCCTCAGAGCACGTTGGCTCAGCTGGTCA
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GTGTGGATGACACACTGGGACAAGTTGGGGCTGTGAAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGA
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TCTGATATCAAGTCAAAATCAGTTTGAAAAGAAGGTGTATCATATTTTATATTGTCACTAGAATCTCTTA
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ACAACTTTGAAGGCAACAGAAGACACTGTTTATTCAAGTCAGTTCTTTGTCAGGTTCCTGCTGTTCTCCT
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ATGACTTTTTGTCAGTTGTTTTGCATTAAAAATATGGCATGCCTAAGATAAAATTGTATATTTTTTCCAT
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TACATTTTATTTGGGGAGGAACATGTGGCTGAGCAGACTTTTGTATAATATTACTTCAAAGATATGTAAT
CACAAACAAAAAAAACTATTTTTTATAATGTCATTTGAGAGAGTTTCATCAGTACAGTTGGTGGACGTTA
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CTTAAATAATCTTAGAATTAATTGATAAATGTCTCTTTTAAAATCTACTGTATTTATTATAATTTACACC
CTTGAAGGTGATCTCTTGTTTTGTGTTGTAAATATATTGTTTGTATGTTTCCCTTCTTGCCTTCTGTTAT
AAGTCTCTTCCTTTCTCAAATAAAGTTTTTTTTAAAAG

HDAC8

SEQ ID NO:103

>gi|8923768|ref|NM_018486.1| Homo sapiens histone deacetylase 8 (HDAC8), mRNA
GCCAGATCTGGAAGGTGGCTGCGGAACGGTTTTAAGCGGAAGATGGAGGAGCCGGAGGAACCGGCGGACA
GTGGGCAGTCGCTGGTCCCGGTTTATATCTATAGTCCCGAGTATGTCAGTATGTGTGACTCCCTGGCCAA
GATCCCCAAACGGGCCAGTATGGTGCATTCTTTGATTGAAGCATATGCACTGCATAAGCAGATGAGGATA
GTTAAGCCTAAAGTGGCCTCCATGGAGGAGATGGCCACCTTCCACACTGATGCTTATCTGCAGCATCTCC
AGAAGGTCAGCCAAGAGGGCGATGATGATCATCCGGACTCCATAGAATATGGGCTAGGTTATGACTGCCC
AGCCACTGAAGGGATATTTGACTATGCAGCAGCTATAGGAGGGGCTACGATCACAGCTGCCCAATGCCTG
ATTGACGGAATGTGCAAAGTAGCAATTAACTGGTCTGGAGGGTGGCATCATGCAAAGAAAGATGAAGCAT

CTGGTTTTTGTTATCTCAATGATGCTGTCCTGGGAATATTACGATTGCGACGGAAATTTGAGCGTATTCT
CTACGTGGATTTGGATCTGCACCATGGAGATGGTGTAGAAGACGCATTCAGTTTCACCTCCAAAGTCATG
ACCGTGTCCCTGCACAAATTCTCCCCAGGATTTTTCCCAGGAACAGGTGACGTGTCTGATGTTGGCCTAG
GGAAGGGACGGTACTACAGTGTAAATGTGCCCATTCAGGATGGCATACAAGATGAAAAATATTACCAGAT
CTGTGAAAGTGTACTAAAGGAAGTATACCAAGCCTTTAATCCCAAAGCAGTGGTCTTACAGCTGGGAGCT
GACACAATAGCTGGGGATCCCATGTGCTCCTTTAACATGACTCCAGTGGGAATTGGCAAGTGTCTTAAGT
ACATCCTTCAATGGCAGTTGGCAACACTCATTTTGGGAGGAGGAGGCTATAACCTTGCCAACACGGCTCG
ATGCTGGACATACTTGACCGGGGTCATCCTAGGGAAAACACTATCCTCTGAGATCCCAGATCATGAGTTT
TTCACAGCATATGGTCCTGATTATGTGCTGGAAATCACGCCAAGCTGCCGGCCAGACCGCAATGAGCCCC
ACCGAATCCAACAAATCCTCAACTACATCAAAGGGAATCTGAAGCATGTGGTCTAGTTGACAGAAAGAGA
TCAGGTTTCCAGAGCTGAGGAGTGGTGCCTATAATGAAGACAGCGTGTTTATGCAAGCAGTTTGTGGAAT
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GCTTCCTGGGGTGAAGAGGCAGGCACCCCAGAGTCCTCAACTGGACCTAGGGGAAGAAGGAGATATCCCA
CATTTAAAGTTCTTATTTAAAAAAACACACACACACAAATGAAATTTTTAATCTTTGAAAATTATTTTTA
AGCGAATTGGGGAGGGGAGTATTTTAATCATCTTAAATGAAACAGATCAGAAGCTGGATGAGAGCAGTCA
CCAGTTTGTAGGGCAGGAGGCAGCTGAGAGGCAGGGTTTGGGCCTCAGGACCATCCAGGTGGAGCCCTGG
GAGAGAGGGTACTGATCAGCAGACTGGGAGGTGGGGAGAAGTCCGCTGGTGTTGTTTTAGTGTTATATAT
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hSIRT2

SEQ ID NO:104

>gi|13775599|ref|NM_012237.2| Homo sapiens sirtuin silent mating type information regulation 2 homolog 2 (S. cerevisiae) (SIRT2), transcript variant 1, mRNA
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ACTCCCATTTCTGGTGCCGTCACGGGACAGAGCAGTCGGTGACAGGACAGAGCAGTCGGTGACGGGACAC
AGTGGTTGGTGACGGGACAGAGCGGTCGGTGACAGCCTCAAGGGCTTCAGCACCGCGCCCATGGCAGAGC
CAGACCCCTCTCACCCTCTGGAGACCCAGGCAGGGAAGGTGCAGGAGGCTCAGGACTCAGATTCAGACTC
TGAGGGAGGAGCCGCTGGTGGAGAAGCAGACATGGACTTCCTGCGGAACTTATTCTCCCAGACGCTCAGC
CTGGGCAGCCAGAAGGAGCGTCTGCTGGACGAGCTGACCTTGGAAGGGGTGGCCCGGTACATGCAGAGCG
AACGCTGTCGCAGAGTCATCTGTTTGGTGGGAGCTGGAATCTCCACATCCGCAGGCATCCCCGACTTTCG
CTCTCCATCCACCGGCCTCTATGACAACCTAGAGAAGTACCATCTTCCCTACCCAGAGGCCATCTTTGAG
ATCAGCTATTTCAAGAAACATCCGGAACCCTTCTTCGCCCTCGCCAAGGAACTCTATCCTGGGCAGTTCA
AGCCAACCATCTGTCACTACTTCATGCGCCTGCTGAAGGACAAGGGGCTACTCCTGCGCTGCTACACGCA
GAACATAGATACCCTGGAGCGAATAGCCGGGCTGGAACAGGAGGACTTGGTGGAGGCGCACGGCACCTTC
TACACATCACACTGCGTCAGCGCCAGCTGCCGGCACGAATACCCGCTAAGCTGGATGAAAGAGAAGATCT
TCTCTGAGGTGACGCCCAAGTGTGAAGACTGTCAGAGCCTGGTGAAGCCTGATATCGTCTTTTTTGGTGA
GAGCCTCCCAGCGCGTTTCTTCTCCTGTATGCAGTCAGACTTCCTGAAGGTGGACCTCCTCCTGGTCATG
GGTACCTCCTTGCAGGTGCAGCCCTTTGCCTCCCTCATCAGCAAGGCACCCCTCTCCACCCCTCGCCTGC
TCATCAACAAGGAGAAAGCTGGCCAGTCGGACCCTTTCCTGGGGATGATTATGGGCCTCGGAGGAGGCAT
GGACTTTGACTCCAAGAAGGCCTACAGGGACGTGGCCTGGCTGGGTGAATGCGACCAGGGCTGCCTGGCC
CTTGCTGAGCTCCTTGGATGGAAGAAGGAGCTGGAGGACCTTGTCCGGAGGGAGCACGCCAGCATAGATG
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TCCTCAGGGACAGCTGAGCCCCAACCGGGCCTGGCCCCCTCTTAACCAGCAGTTCTTGTCTGGGGAGCTC
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AGCAAATCTCTAACACCTCCTAGAGGCCAAGGCTTAAACAGGCATCTCTACCAGCCCCACTGTCTCTAAC
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TTTAACTCTTCCAGGACAGGGAGCTTCGGGCCCCCACTCTGTCTCCTGCCCCCGGGGGCCTGTGGCTAAG
TAAACCATACCTAACCTACCCCAGTGTGGGTGTGGGCCTCTGAATATAACCCACACCCAGCGTAGGGGGA
GTCTGAGCCGGGAGGGCTCCCGAGTCTCTGCCTTCAGCTCCCAAAGTGGGTGGTGGGCCCCCTTCACGTG
GGACCCACTTCCCATGCTGGATGGGCAGAAGACATTGCTTATTGGAGACAAATTAAAAACAAAAACAACT
AAC

>gi|13775601|ref|NM_030593.1| Homo sapiens sirtuin silent mating type information regulation 2 homolog 2 (S. cerevisiae) (SIRT2), transcript variant 2, mRNA SEQ ID NO:105
CGAAAGCGCGTCTGCGGCCGCAATGTCTGCTGAGAGTTGTAGTTCTGTGCCCTATCACGGCCACTCCCAT
TTCTGGTGCCGTCACGGGACAGAGCAGTCGGTGACAGGACAGAGCAGTCGGTGACGGGACACAGTGGTTG
GTGACGGGACAGAGCGGTCGGTGACAGCCTCAAGGGCTTCAGCACCGCGCCCATGGCAGAGCCAGACCGA
CTCAGATTCAGACTCTGAGGGAGGAGCCGCTGGTGGAGAAGCAGACATGGACTTCCTGCGGAACTTATTC
TCCCAGACGCTCAGCCTGGGCAGCCAGAAGGAGCGTCTGCTGGACGAGCTGACCTTGGAAGGGGTGGCCC
GGTACATGCAGAGCGAACGCTGTCGCAGAGTCATCTGTTTGGTGGGAGCTGGAATCTCCACATCCGCAGG

CATCCCCGACTTTCGCTCTCCATCCACCGGCCTCTATGACAACCTAGAGAAGTACCATCTTCCCTACCCA
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ATCCTGGGCAGTTCAAGCCAACCATCTGTCACTACTTCATGCGCCTGCTGAAGGACAAGGGGCTACTCCT
GCGCTGCTACACGCAGAACATAGATACCCTGGAGCGAATAGCCGGGCTGGAACAGGAGGACTTGGTGGAG
GCGCACGGCACCTTCTACACATCACACTGCGTCAGCGCCAGCTGCCGGCACGAATACCCGCTAAGCTGGA
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CCACCCCTCGCCTGCTCATCAACAAGGAGAAAGCTGGCCAGTCGGACCCTTTCCTGGGGATGATTATGGG
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CCCAGCGTAGGGGGAGTCTGAGCCGGGAGGGCTCCCGAGTCTCTGCCTTCAGCTCCCAAAGTGGGTGGTG
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hSIRT3

SEQ ID NO:106

gi|13775603|ref|NM_012239.3| Homo sapiens sirtuin silent mating type information regulation 2 homolog 3 (S. cerevisiae) (SIRT3), mRNA

GGCGCCGGGGGCGGGGGTGGGAGGCGGAGGCGGGGCCGGGGCGCCGCGGGCGGGGCGCCGGGGGCGGGGC
GAGTCCGGAGGACTCCTCGGACTGCGCGGAACATGGCGTTCTGGGGTTGGCGCGCCGCGGCAGCCCTCCG
GCTGTGGGGCCGGGTAGTTGAACGGGTCGAGGCCGGGGGAGGCGTGGGGCCGTTTCAGGCCTGCGGCTGT
CGGCTGGTGCTTGGCGGCAGGGACGATGTGAGTGCGGGGCTGAGAGGCAGCCATGGGGCCCGCGGTGAGC
CCTTGGACCCGGCGCGCCCCTTGCAGAGGCCTCCCAGACCCGAGGTGCCCAGGGCATTCCGGAGGCAGCC
GAGGGCAGCAGCTCCCAGTTTCTTCTTTTCGAGTATTAAAGGTGGAAGAAGGTCCATATCTTTTTCTGTG
GGTGCTTCAAGTGTTGTTGGAAGTGGAGGCAGCAGTGACAAGGGGAAGCTTTCCCTGCAGGATGTAGCTG
AGCTGATTCGGGCCAGAGCCTGCCAGAGGGTGGTGGTCATGGTGGGGGCCGGCATCAGCACACCCAGTGG
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CTTTGGGGAGCCGCTGCCCCAGAGGTTCTTGCTGCATGTGGTTGATTTCCCCATGGCAGATCTGCTGCTC
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GACTGCTCATCAACCGGGACTTGGTGGGGCCCTTGGCTTGGCATCCTCGCAGCAGGGACGTGGCCCAGCT
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GTAACATAGGAGACATCCACATCCCAATTCTGACAAGACCTCATGCCTGAAGACAGCTTGGGCAGGTGAA
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TGACTGGTAGGGCTGTGTTTACAGTCAGGGCCACCCCGTCACATATACAAAGGAGCTGCCTGCCTGTTTG
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TCTGTTGGTTACAAGATCCAGACTTGGGCCGAGCGGTCCCCAGCCCTCTTCATGTTCCGAAGTGTAGTCT
TGAGGCCCTGGTGCCGCACTTCTAGCATGTTGGTCTCCTTTAGTGGGGCTATTTTTAATGAGAGAAAATC
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hSIRT4

SEQ ID NO:107

>gi|6912661|ref|NM_012240.1| Homo sapiens sirtuin silent mating type information regulation 2 homolog 4 (S. cerevisiae) (SIRT4), mRNA
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CGCAAACCCCAGCCAGCCGTGCTCGAAAGCCTCCATTGGGTTATTTGTGCCAGCAAGTCCTCCTCTGGAC
CCTGAGAAGGTCAAAGAGTTACAGCGCTTCATCACCCTTTCCAAGAGACTCCTTGTGATGACTGGGGCAG
GAATCTCCACCGAATCGGGGATACCAGACTACAGGTCAGAAAAAGTGGGGCTTTATGCCCGCACTGACCG
CAGGCCCATCCAGCATGGTGATTTTGTCCGGAGTGCCCCAATCCGCCAGCGGTACTGGGCGAGAAACTTC
GTAGGCTGGCCTCAATTCTCCTCCCACCAGCCTAACCCTGCACACTGGGCTTTGAGCACCTGGGAGAAAC
TCGGAAAGCTGTACTGGTTGGTGACCCAAAATGTGGATGCTTTGCACACCAAGGCGGGGAGTCGGCGCCT
GACAGAGCTCCACGGATGCATGGACAGGGTCCTGTGCTTGGATTGTGGGGAACAGACTCCCCGGGGGGTG
CTGCAAGAGCGTTTCCAAGTCCTGAACCCCACCTGGAGTGCTGAGGCCCATGGCCTGGCTCCTGATGGTG
ACGTCTTTCTCTCAGAGGAGCAAGTCCGGAGCTTTCAGGTCCCAACCTGCGTTCAATGTGGAGGCCATCT
GAAACCAGATGTCGTTTTCTTCGGGGACACAGTGAACCCTGACAAGGTTGATTTTGTGCACAAGCGTGTA
AAAGAAGCCGACTCCCTCTTGGTGGTGGGATCATCCTTGCAGGTATACTCTGGTTACAGGTTTATCCTCA
CTGCCTGGGAGAAGAAGCTCCCGATTGCAATACTGAACATTGGGCCCACACGGTCGGATGACTTGGCGTG
TCTGAAACTGAATTCTCGTTGTGGAGAGTTGCTGCCTTTGATAGACCCATGCTGACCACAGCCTGATATT
CCAGAACCTGGAACAGGGACTTTCACTTGAATCTTGCTGCTAAATGTAAATGCCTTCTCAAATGACAGAT
TCCAGTTCCCATTCAACAGAGTAGGGTGCACTGACAAAGTATAGAAGGTTCTAGGTATCTTAATGTGTGG
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hSIRT5

SEQ ID NO:108

>gi|13787213|ref|NM_012241.2| Homo sapiens sirtuin silent mating type information regulation 2 homolog 5 (S. cerevisiae) (SIRT5), transcript variant 1, mRNA
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AGAAGAAAATAATTTTCTGGATCAAATTAGAAGTCTGTATTATATTGATGTCTCCAGATTCAAATATATT
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CTACAGACAAACCCTGATGCGACCTCTCCAGATTGTCCCAAGTCGATTGATTTCCCAGCTATATTGTGGC
CTGAAGCCTCCAGCGTCCACACGAAACCAGATTTGCCTGAAAATGGCTCGGCCAAGTTCAAGTATGGCAG
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>gi|13787214|ref|NM_031244.1| Homo sapiens sirtuin silent mating type information regulation 2 homolog 5 (S. cerevisiae) (SIRT5), transcript variant 2, mRNA SEQ ID NO:109
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hSIRT6

SEQ ID NO:110

>gi|7706709|ref|NM_016539.1| Homo sapiens sirtuin silent mating type information regulation 2 homolog 6 (S. cerevisiae) (SIRT6), mRNA
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hSIRT7

SEQ ID NO:111

>gi|7706711|ref|NM_016538.1| Homo sapiens sirtuin silent mating type information regulation 2 homolog 7 (S. cerevisiae) (SIRT7), mRNA

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MECP2

SEQ ID NO:112

>gi|7710148|ref|NM_004992.2| Homo sapiens methyl CpG binding protein 2 (Rett syndrome) (MECP2), mRNA

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CGAGAGCTATGGACAGCATTGCCAGTAACACAGGCCACCCTGTGCAGAAGGGAGCTGGCTCCAGCCTGGA
AACCTGTCTGAGGTTGGGAGAGGTGCACTTGGGGCACAGGGAGAGGCCGGGACACACTTAGCTGGAGATG
TCTCTAAAAGCCCTGTATCGTATTCACCTTCAGTTTTTGTGTTTTGGGACAATTACTTTAGAAAATAAGT
AGGTCGTTTTAAAAACAAAAATTATTGATTGCTTTTTTGTAGTGTTCAGAAAAAAGGTTCTTTGTGTATA
GCCAAATGACTGAAAGCACTGATATATTTAAAAACAAAAGGCAATTTATTAAGGAAATTTGTACCATTTC
AGTAAACCTGTCTGAATGTACCTGTATACGTTTCAAAAACACCCCCCCCCCACTGAATCCCTGTAACCTA
TTTATTATATAAAGAGTTTGCCTTATAAATTT

ZNF145

SEQ ID NO:113

>gi|5174752|ref|NM_006006.1| Homo sapiens zinc finger protein 145 (Kruppel-like, expressed in promyelocytic leukemia) (ZNF145), mRNA

CAGGAAGCCCACCCAGCCCCGCCACGCAGAGCCCAGAAGGAAAGAAAGCCTCATGCCTGAGCCGAGGGGA
GCACCATGGATCTGACAAAAATGGGCATGATCCAGCTGCAGAACCCTAGCCACCCCACGGGGCTACTGTG

CAAGGCCAACCAGATGCGGCTGGCCGGGACTTTGTGCGATGTGGTCATCATGGTGGACAGCCAGGAGTTC
CACGCCCACCGGACGGTGCTGGCCTGCACCAGCAAGATGTTTGAGATCCTCTTCCACCGCAATAGTCAAC
ACTATACTTTGGACTTCCTCTCGCCAAAGACCTTCCAGCAGATTCTGGAGTATGCATATACAGCCACGCT
GCAAGCCAAGGCGGAGGACCTGGATGACCTGCTGTATGCGGCCGAGATCCTGGAGATCGAGTACCTGGAG
GAACAGTGCCTGAAGATGCTGGAGACCATCCAGGCCTCAGACGACAATGACACGGAGGCCACCATGGCCG
ATGGCGGGGCCGAGGAAGAAGAGGACCGCAAGGCTCGGTACCTCAAGAACATCTTCATCTCGAAGCATTC
CAGCGAGGAGAGTGGGTATGCCAGTGTGGCTGGACAGAGCCTCCCTGGGCCCATGGTGGACCAGAGCCCT
TCAGTCTCCACTTCATTTGGTCTTTCAGCCATGAGTCCCACCAAGGCTGCAGTGGACAGTTTGATGACCA
TAGGACAGTCTCTCCTGCAGGGAACTCTTCAGCCACCTGCAGGGCCCGAGGAGCCAACTCTGGCTGGGGG
TGGGCGGCACCCTGGGGTGGCTGAGGTGAAGACGGAGATGATGCAGGTGGATGAGGTGCCCAGCCAGGAC
AGCCCTGGGGCAGCCGAGTCCAGCATCTCAGGAGGGATGGGGGACAAGGTTGAGGAAAGAGGCAAAGAGG
GGCCTGGGACCCCGACTCGAAGCAGCGTCATCACCAGTGCTAGGGAGCTACACTATGGGCGAGAGGAGAG
TGCCGAGCAGGTGCCACCCCCAGCTGAGGCTGGCCAGGCCCCCACTGGCCGACCTGAGCACCCAGCACCC
CCGCCTGAGAAGCATCTGGGCATCTACTCCGTGTTGCCCAACCACAAGGCTGACGCTGTATTGAGCATGC
CGTCTTCCGTGACCTCTGGCCTCCACGTGCAGCCTGCCCTGGCTGTCTCCATGGACTTCAGCACCTATGG
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AAGTCAGAGAGCCGGACCATCGGAGAGCAGTGCAGCGTGTGTGGGGTCGAGCTTCCTGATAACGAGGCTG
TGGAGCAGCACAGGAAGCTGCACAGTGGGATGAAGACGTACGGGTGCGAGCTCTGCGGGAAGCGGTTCCT
GGATAGTTTGCGGCTGAGAATGCACTTACTGGCTCATTCAGCGGGTGCCAAAGCCTTTGTCTGTGATCAG
TGCGGTGCACAGTTTTCGAAGGAGGATGCCCTGGAGACACACAGGCAGACCCATACTGGCACTGACATGG
CCGTCTTCTGTCTGCTGTGTGGGAAGCGCTTCCAGGCGCAGAGCGCACTGCAGCAGCACATGGAGGTCCA
CGCGGGCGTGCGCAGCTACATCTGCAGTGAGTGCAACCGCACCTTCCCCAGCCACACGGCTCTCAAACGC
CACCTGCGCTCACATACAGGCGACCACCCCTACGAGTGTGAGTTCTGTGGCAGCTGCTTCCGGGATGAGA
GCACACTCAAGAGCCACAAACGCATCCACACGGGTGAGAAACCCTACGAGTGCAATGGCTGTGACAAGAA
GTTCAGCCTCAAGCATCAGCTGGAGACGCACTATAGGGTGCACACAGGTGAGAAGCCCTTTGAGTGTAAG
CTCTGCCACCAGCGCTCCCGGGACTACTCGGCCATGATCAAGCACCTGAGAACGCACAACGGCGCCTCGC
CCTACCAGTGCACCATCTGCACAGAGTACTGCCCCAGCCTCTCCTCCATGCAGAAGCACATGAAGGGCCA
CAAGCCCGAGGAGATCCCGCCCGACTGGAGGATAGAGAAGACGTACCTCTACCTGTGCTATGTGTGAAGG
GAGGCCCGCGGCGGTGGAGCCGAGCGGGGAGCCAGGAAAGAAGAGTTGGAGTGAGATGAAGGAAGGACTA

TFDP1

SEQ ID NO:114

>gi|6005899|ref|NM_007111.1| Homo sapiens transcription factor Dp-1 (TFDP1), mRNA
GGAATTCCGTAGCTATTGATTTCCCGGATCTGGTAACATGGCAAAAGATGCCGGTCTAATTGAAGCCAAC
GGAGAACTCAAGGTCTTCATAGACCAGAACCTTAGTCCCGGGAAAGGCGTGGTGTCCCTCGTGGCCGTTC
ACCCCTCCACCGTCAACCCGCTCGGGAAGCAGCTCTTGCCAAAAACCTTTGGACAGTCCAATGTCAACAT
TGCCCAGCAAGTGGTAATTGGTACGCCTCAGAGACCGGCAGCGTCAAACACCCTGGTGGTAGGAAGCCCA
CACACCCCCAGCACTCACTTTGCCTCTCAGAACCAGCCTTCCGACTCCTCACCTTGGTCTGCCGGGAAGC
GCAACAGGAAAGGAGAGAAGAATGGCAAGGGCCTACGGCATTTCTCCATGAAGGTCTGCGAGAAGGTGCA
GAGGAAAGGGACCACTTCCTACAACGAAGTGGCAGACGAGCTGGTTGCGGAGTTCAGTGCTGCCGACAAC
CACATCTTACCAAACGAGTCAGCTTATGACCAGAAAAACATAAGACGGCGCGTCTACGATGCCTTAAACG
TGCTAATGGCCATGAACATCATCTCCAAGGAGAAGAAGGAGATCAAGTGGATTGGTCTGCCCACCAACTC
GGCTCAGGAATGTCAGAACTTAGAGGTGGAAAGACAGAGGAGACTTGAAAGAATAAAACAGAAACAGTCT
CAACTTCAAGAACTTATTCTACAGCAAATTGCCTTCAAGAACCTGGTGCAGAGAAACCGGCATGCGGAGC
AGCAGGCCAGCCGGCCACCGCCACCCAACTCAGTCATCCACCTGCCCTTCATCATCGTCAACACCAGCAA
GAAGACGGTCATCGACTGCAGCATCTCCAATGACAAATTTGAGTATCTGTTTAATTTTGACAACACATTT
GAAATCCACGATGACATAGAAGTGCTGAAGCGGATGGGCATGGCTTGCGGGCTGGAGTCGGGGAGCTGCT
CTGCCGAAGACCTTAAAATGGCCAGAAGTCTGGTCCCCAAGGCTCTGGAGCCATACGTGACAGAAATGGC
TCAGGGAACTGTTGGAGGCGTGTTCATCACGACGGCAGGTTCCACGTCTAACGGCACAAGGTTCTCTGCC
AGTGACCTGACCAACGGTGCAGATGGGATGCTGGCCACAAGCTCCAATGGGTCTCAGTACAGCGGCTCCA
GGGTGGAGACTCCGGTGTCCTACGTCGGGGAGGACGACGAGGAGGACGATGACTTCAACGAGAATGACGA
GGACGACTGACGTCCTCCCCACTTCAGATTCGGCTTCAGGAAAACGTTTAGCGAAAAGAAACTTTTTTTT
TAATGTGGGTTTTCTGTTTCCTTTTGGCCTAGTCCCAAGAAGATATTGGTAAGCTATTGAATTTAGATAT
GCACCTCTGATAAGCAAGGATTGTTTCCCGTAGATTAGGA

SAP30

SEQ ID NO:115

>gi|4506782|ref|NM_003864.1| Homo sapiens sin3-associated polypeptide, 30kD (SAP30), mRNA

CCCCCATGTGACAGTGACGGGGTCCCCGCTCCAGGAGACGCTCGAGTCTGCGTCCCGGCCCTCAGCACTG
TCCACTGTTTCGGTGCCAGCAGAGACCAGCAGGCCCGGGACAGTTGGTGTTTGGCCGTGCCGCTGTCTAA
CTTGGTGTGCAGAGTGAATTGCCGCTGCCGGAGCGGAGAGAGGCGGAGCGGCCAGGAGAGAGGGGATTTC
TGTCAGCGCCGGCCTCGGGAGCTCGGAGACATGAACGGCTTCACGCCTGACGAGATGAGCCGCGGCGGGG
ATGCGGCCGCCGCAGTGGCCGCAGTGGTCGCTGCCGCGGCCGCCGCCGCCTCGGCGGGGAACGGGACCGG
CGCGGGCACCGGGGCTGAGGTGCCGGGCGCGGGGGCGGTCTCAGCGGCTGGGCCCCCGGGGGCGGCCGGG
CCGGGCCCCGGGCAACTGTGCTGCCTGCGGGAGGATGGTGAGCGGTGCGGCCGGGCGGCAGGCAACGCCA
GCTTCAGCAAGAGGATCCAGAAGAGCATCTCCCAGAAGAAGGTGAAGATCGAGCTGGATAAGAGCGCAAG
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AGTGATGATGATGGAGGTGATTCACCTGTTCAAGATATTGATACCCCAGAGGTTGATTTATACCAATTAC
AAGTAAATACACTTAGGAGATACAAAAGACACTTCAAGCTACCAACCAGACCAGGACTTAATAAAGCACA
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GGAATTGAGACTAATAACTTGGATGTTAACACTGTTTACTGTTTTTTCACATGTAGAAATGTTCTTTGTG
TATTTTTTCTACAGAGGATTTTCTCTGATTTTATTTTCTTTGTTTCTGACTCTAATAATTAGTTGGAAAC
TCATATAAAATGAGCTTTCCTAAATTAAATCTATTTTAAATAAAGGTTATTACTATTAAAAAAAAAAAAA
AAAAA

SAP18

SEQ ID NO:116

>gi|12056471|ref|NM_005870.2| Homo sapiens sin3-associated polypeptide, 18kD (SAP18), mRNA

AGGTTTGAACTGACTTTATTATTTTGTAAATGTGAATTTTACAAAGCGCTTTACAATTAATGATCACATC
CTTTTGTTTGTCATGGATTTCCACTGTCTGAAACGGCTCTGAGCACGCTTGAAGCCCTCGGTTTCCCTGT
TCGCTTTTGAATGTTTCAGTTTTAGTTATTGATACAATGTCAGCCATGGCTAAAAAGTAACAGTCTTGAC
TCTACCGAGTAACAGCACAAAAACAGAGTGAGGGCTCAGGAAAACAAAACAAAGGCTTCCTCCTTAAAAA
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ACAGCACAGGAAAATTTATTTTTTAACAGTCGTGAGTTACAGTACTTTAACCCCTAAACAGACTCTTTAA
AACAACCGTCTCCTTTTTAAAAGTCTCTTTTTTCCAAACATTCCATCCGAAGGATGGATGCTCTACTTGC
ACCCAGTGCCATCCAAATGTTCAAGTCAAAAATATTTATACATTTTATACTTAGGCGAGCGTCTCGCAGG
CCGTAGGAGGAAGATGGCGGTGGAGTCGCGCGTTACCCAGGAGGAAATTAAGAAGGAGCCAGAGAAACCG
ATCGACCGCGAGAAGACATGCCCACTGTTGCTACGGGTCTTCACCACCAATAACGGCCGCCACCACCGAA
TGGACGAGTTCTCCCGGGGAAATGTACCGTCCAGCGAGTTGCAGATCTACACTTGGATGGATGCAACTTT
GAAAGAACTGACAAGCTTAGTAAAAGAAGTCTACCCAGAAGCTAGAAAGAAGGGCACTCACTTCAATTTT
GCAATCGTTTTTACAGATGTTAAAAGACCTGGCTATCGAGTTAAGGAGATTGGCAGCACCATGTCTGGCA
GAAAGGGGACTGATGATTCCATGACCCTGCAGTCGCAGAAGTTCCAGATAGGAGATTACTTGGACATAGC
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AAGCCTTTAAATTCTAAACAAATTATAATGCATCATCTATTTAGGAGTTAGATTTGGATGTGCTATTGTA
TGATTACGAATAGTCTGTATGTTTCAAGCCCTTCTGTAAAATATGAAGAAAAGTCTCTTAGCATTCTGTG
TAAAACTGTACTGTTAAATATATGTGTGTAATC

RBBP7

SEQ ID NO:117

>gi|13259504|ref|NM_002893.2| Homo sapiens retinoblastoma binding protein 7 (RBBP7), mRNA

GCCTCGTCAGCTGCCTGGGCGGGCTGGGAGGCGCGGGTTGAAAAGTCTCGTTCCAAGTTTGGAGAGAGAG
AGAAGAGCGCCTCAGACCTCGGTACCCGCGAGCGGGGAGGAGGCAGGAAAGAAGGACGCGGCGTCTGGGG
AGCACCCAGGCAGCAAGACGGGGCCCGGGCTTTCGACAGTGGGGAGTGTGACGCGCTTGGGAAAGGCAGG
AGCGCCAGCGGTCGGGCTGCTCTTGGCTAACGAGAGGAGTCCGAGGCGGCGGCGAGGGGCGAACGACCCG
ACGCAAGATGGCGAGTAAAGAGATGTTTGAAGATACTGTGGAGGAGCGTGTCATCAATGAAGAATATAAA
ATCTGGAAGAAGAATACACCGTTTCTATATGACCTGGTTATGACCCATGCTCTTCAGTGGCCCAGTCTTA
CCGTTCAGTGGCTTCCTGAAGTGACTAAACCTGAAGGAAAAGATTATGCCCTTCATTGGCTAGTGCTGGG
GACTCATACGTCTGATGAGCAGAATCATCTGGTGGTTGCTCGAGTACATATTCCCAATGATGATGCACAG
TTTGATGCTTCCCATTGTGACAGTGACAAGGGTGAATTTGGTGGCTTTGGTTCTGTAACAGGAAAAATTG

AATGTGAAATTAAAATCAATCACGAAGGAGAAGTAAACCGTGCTCGTTACATGCCGCAGAATCCTCACAT
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CCAAGTGGAGAATGTAATCCTGATCTCAGATTAAGAGGTCACCAGAAGGAAGGCTATGGTCTCTCCTGGA
ATTCAAATTTGAGTGGACATCTCCTAAGTGCATCTGATGACCATACTGTTTGTCTGTGGGATATAAACGC
AGGACCAAAAGAAGGCAAAATTGTGGATGCTAAAGCCATCTTTACTGGCCACTCAGCTGTTGTAGAGGAT
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ACACCAGGTCCAATACCACCTCCAAGCCGAGTCACTTGGTGGATGCGCACACTGCCGAAGTCAACTGCCT
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CACACTGCTAAGATTTCAGATTTTAGCTGGAACCCCAATGAGCCTTGGGTCATTTGCTCAGTGTCTGAGG
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AGAAGAATGTTGCTTAAGAAAGAACATCACCATTGATTTTAAATACAAGTAGCAGGGTATTGCCTTTGAT
TCAACTGTTTTAAGTCCTCATTTTCTCAAACTAAGTGCTTGCTGTTCCCAAATATGCAAGAATAACTTTT
ACACTTTTTCCTTCCAACACTTCTTGATTGGCTTTGCAGAAATAAAGTTTTAAAAT

RBBP4

SEQ ID NO:118

>gi|5032026|ref|NM_005610.1| Homo sapiens retinoblastoma binding protein 4 (RBBP4), mRNA
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CTCGCCTGCCCGCCATGGCCGACAAGGAAGCAGCCTTCGACGACGCAGTGGAAGAACGAGTGATCAACGA
GGAATACAAAATATGGAAAAAGAACACCCCTTTTCTTTATGATTTGGTGATGACCCATGCTCTGGAGTGG
CCCAGCCTAACTGCCCAGTGGCTTCCAGATGTAACCAGACCAGAAGGGAAAGATTTCAGCATTCATCGAC
TTGTCCTGGGGACACACACATCGGATGAACAAAACCATCTTGTTATAGCCAGTGTGCAGCTCCCTAATGA
TGATGCTCAGTTTGATGCGTCACACTACGACAGTGAGAAAGGAGAATTTGGAGGTTTTGGTTCAGTTAGT
GGAAAAATTGAAATAGAAATCAAGATCAACCATGAAGGAGAAGTAAACAGGGCCCGTTATATGCCCCAGA
ACCCTTGTATCATCGCAACAAAGACTCCTTCCAGTGATGTTCTTGTCTTTGACTATACAAAACATCCTTC
TAAACCAGATCCTTCTGGAGAGTGCAACCCAGACTTGCGTCTCCGTGGACATCAGAAGGAAGGCTATGGG
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ACATCAGTGCCGTTCCAAAGGAGGGAAAAGTGGTAGATGCGAAGACCATCTTTACAGGGCATACGGCAGT
AGTAGAAGATGTTTCCTGGCATCTACTCCATGAGTCTCTGTTTGGGTCAGTTGCTGATGATCAGAAACTT
ATGATTTGGGATACTCGTTCAAACAATACTTCCAAACCAAGCCACTCAGTTGATGCTCACACTGCTGAAG
TGAACTGCCTTTCTTTCAATCCTTATAGTGAGTTCATTCTTGCCACAGGATCAGCTGACAAGACTGTTGC
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TAAGTAAAGGCTAGAGTGAGTAAGGAATAGAGCCAAATGAGGTAGGTGTCTGAGCCATGAAGTATAAATA
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AGGCCTGTTACTCTCCATGACTAACTGTGTAAGTGCTTAAAATGGAATAAATTGCTTTTCTACATAAAAA
AAAA

RB1

SEQ ID NO:119

gi|4506434|ref|NM_000321.1| Homo sapiens retinoblastoma 1 (including osteosarcoma) (RB1), mRNA

TTCCGGTTTTTCTCAGGGGACGTTGAAATTATTTTTGTAACGGGAGTCGGGAGAGGACGGGGCGTGCCCC
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GCCGCCCAAAACCCCCCGAAAAACGGCCGCCACCGCCGCCGCTGCCGCCGCGGAACCCCCGGCACCGCCG
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TTGGAAAGGACATGTGAACTTATATATTTGACACAACCCAGCAGTTCGATATCTACTGAAATAAATTCTG
CATTGGTGCTAAAAGTTTCTTGGATCACATTTTTATTAGCTAAAGGGGAAGTATTACAAATGGAAGATGA
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AGATATTATTGCTTTATTGCTTTTTTGTATTGGTTAAAACTGTACATTTAAAATTGCTATGTTACTATTT
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MEN1

SEQ ID NO:120

>gi|4557744|ref|NM_000244.1| Homo sapiens multiple endocrine neoplasia I (MEN1), mRNA
GGTGTCCGGAGCCGCGGACCTAGAGATCCCAGAAGCCACAGCGCAGCGGCCCGGCCCGCCACTATTTCCA
GGCTCTGCGGGGCAGGGGCCGCCGCCCACCGCCCGCCGCCATGGGGCTGAAGGCCGCCCAGAAGACGCTG
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Table 5 Histone Acetyltranferases.

GCN5/PCAF Family.

Gcn5

SEQ ID NO:121

>gi|4503954|ref|NM_001487.1| Homo sapiens GCN5 general control of amino-acid synthesis 5-like 1 (yeast) (GCN5L1), mRNA

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GCN5L2

SEQ ID NO:122

>gi|10835100|ref|NM_021078.1| Homo sapiens GCN5 general control of amino-acid synthesis 5-like 2 (yeast) (GCN5L2), mRNA

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CAGCCCAAACTGAAGGGGGCCATGCCTTGTCCAGCACTGTTCTGTCAGTCTCCCCCAGGGGTGGGGGGTA
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PCAF

SEQ ID NO:123

>gi|6382075|ref|NM_003884.2| Homo sapiens p300/CBP-associated factor (PCAF), mRNA

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MOZ

SEQ ID NO:124

>gi|5803097|ref|NM_006766.1| Homo sapiens zinc finger protein 220

(ZNF220), mRNA
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HBO1

SEQ ID NO:125

>gi|5901961|ref|NM_007067.1| Homo sapiens histone acetyltransferase (HBOA), mRNA

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SRC Family

SRC-1

SEQ ID NO:126

>gi|4507198|ref|NM_003743.1| Homo sapiens nuclear receptor coactivator 1 (NCOA1), mRNA

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PCIP

SEQ ID NO:127

>gi|5729725|ref|NM_006534.1| Homo sapiens nuclear receptor coactivator 3 (NCOA3), mRNA
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GRIP1

SEQ ID NO:127

gi|5729857|ref|NM_006540.1| Homo sapiens nuclear receptor coactivator 2 (NCOA2), mRNA

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GGTTATTGCCAAACCAGAATTTACCACTTGACATCACATTGCAAAGCCCAACTGGTGCTGGACCTTTCCC
ACCAATCAGAAACAGTAGTCCCTACTCAGTGATACCTCAGCCAGGAATGATGGGTAATCAAGGGATGATA
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GGCCAAAGACAGACGCTTCAGTCTCAGGTCATGAATATAGGGCCATCTGAATTAGAGATGAACATGGGGG
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AGACCAGGCGTCTTTTGCCAGCCAAAACAGGCAGCCATTTGGCAGTTCTCCAGATGACTTGCTATGTCCA
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ATF-2

SEQ ID NO:128

>gi|4503032|ref|NM_001880.1| Homo sapiens activating transcription factor 2 (ATF2), mRNA

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GNAT related

HAT1

SEQ ID NO:129

>gi|4504340|ref|NM_003642.1| Homo sapiens histone acetyltransferase 1 (HAT1), mRNA

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Table 6 ATP-dependent Chromatin Remodelling.

SMARCA5

SEQ ID NO:130

>gi|4507074|ref|NM_003601.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 (SMARCA5), mRNA

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SMARCA2

SEQ ID NO:131

gi|4507068|ref|NM_003070.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2 (SMARCA2), mRNA

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SMARCA4

SEQ ID NO:132

>gi|4507072|ref|NM_003072.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4), mRNA

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SMARCA3

SEQ ID NO:133

>gi|4507070|ref|NM_003071.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3 (SMARCA3), mRNA

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SMARCAL1

SEQ ID NO:134

>gi|7657149|ref|NM_014140.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a-like 1 (SMARCAL1), mRNA

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SMARCA1

SEQ ID NO:135

>gi|4507066|ref|NM_003069.1| Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1 (SMARCA1), mRNA

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CHRAC1

SEQ ID NO:136
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Claims

1. An RNAi molecule derived from a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence as represented by the sequences in SEQ ID NO's: 7-23, or fragment thereof;

b) a nucleic acid sequence which hybridises to the nucleic acid sequences of SEQ ID NO's: 7-23 and encodes a Notch signalling target gene;

c) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

2. An RNAi molecule according to Claim 1 wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their length and further wherein said first and second parts form a double stranded region by complementary base pairing over at least part of their length.

3. An RNAi molecule according to Claim 2 wherein said first and second parts are linked by at least one nucleotide base.

4. An RNAi molecule according to Claim 3 wherein said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide bases.

5. An RNAi molecule according to Claim 2 wherein said linker is at least 10 nucleotide bases.

6. An RNAi molecule according to any of Claims 1-5 wherein the length of said RNAi molecule is between 10 nucleotide bases (nb) –1000nb.

7 An RNAi molecule according to Claim 6 wherein the length of said RNA molecule is selected from 10nb; 20nb; 30nb; 40nb; 50nb; 60nb; 70nb; 80nb; or 90nb.

8 An RNAi molecule according to Claim 6 wherein said RNA is 21nb in length.

9 An RNAi molecule according to Claim 6 wherein said RNA molecule is 100nb; 200nb; 300nb; 400nb; 500nb; 600nb; 700nb; 800nb; 900nb; or 1000nb in length.

10. An RNAi molecule according to any of Claims 1-5 wherein said RNA molecule is at least 1000nb.

11. An RNAi molecule according to any of Claims 1-10 wherein said RNAi molecule comprise modified nucleotide bases.

12. A nucleic acid molecule encoding at least part of a gene which modulates stem cell differentiation comprising a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence as represented by the sequences in SEQ ID NO: 7-23, or fragment thereof;

b) a nucleic acid sequence which hybridises to the nucleic acid sequences of SEQ ID NO: 7-23 and is a Notch signalling target gene;

c) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii) wherein said nucleic acid molecule comprises a first part linked to a second part which first and second parts are complementary over at least part of their length, which nucleic acid molecule is operably linked to at least one further nucleic acid molecule capable of promoting transcription of said nucleic acid linked thereto and further wherein said first and second parts form a double stranded region by complementary base

pairing over at least part of their length as or when said nucleic acid molecule is transcribed.

13. An expression vector including an expression cassette comprising at least one nucleic acid molecule according to Claim 12.

14. A method of treatment of an animal, preferably a human, comprising administering an effective amount of at least one RNAi molecule according to any of Claims 1-11 or a vector according to Claim 13, to a subject to be treated.

15. An *in vitro* method to modulate the differentiation state of a pluripotential stem cell comprising the steps of:

i) contacting a pluripotential stem cell with at least one inhibitory RNA molecule (RNAi) comprising a sequence of a gene which mediates at least one step in the differentiation of said cell wherein said gene is selected from the group consisting of;

a) a nucleic acid sequence as represented by the sequences in SEQ ID NO: 7-23, or fragment thereof;

b) a nucleic acid sequence which hybridises to the nucleic acid sequences of SEQ ID NO: 7-23 and is a Notch signalling target gene;

c) a nucleic acid sequence which comprise sequences which are degenerate as a result of the genetic code to the nucleic acid sequences defined in (i) and (ii).

(ii) providing conditions conducive to the proliferation of the cell treated in (i) above; and optionally

(iii) maintaining and/or storing said cell.

16. A lineage restricted stem cell or a differentiated stem cell obtainable by the method according to Claim 15.

17. A lineage restricted stem cell according to Claim 16 wherein said cell is selected from the group consisting of: haemopoietic stem cell; neural stem cell; bone stem cell; muscle stem cell; mesenchymal stem cell; trophoblastic stem cell; epithelial stem cell (derived from organs such as the skin, gastrointestinal mucosa, kidney, bladder, mammary glands, uterus, prostate and endocrine glands such as the pituitary); endodermal stem cell (derived from organs such as the liver, pancreas, lung and blood vessels).

18. A differentiated cell according to Claim 16 wherein said cell is selected from the group consisting of: a nerve cell; a mesenchymal cell; a muscle cell (cardiomyocyte); a liver cell; a kidney cell; a blood cell (eg erythrocyte, CD4+ lymphocyte, CD8+ lymphocyte; panceatic β cell; epithelial cell (eg lung, gastric,) ; an endothelial cell.

19. A cell culture comprising at least one lineage restricted stem cell or differentiated cell according to any of Claims 16-18.

20. An organ comprising a lineage restricted stem cell or a differentiated stem cell according to any of Claims 16-18.

21. A method of treatment of an animal, preferably a human, comprising administering a cell or organ according to any of Claims 16-18 or 20.

Figure 1

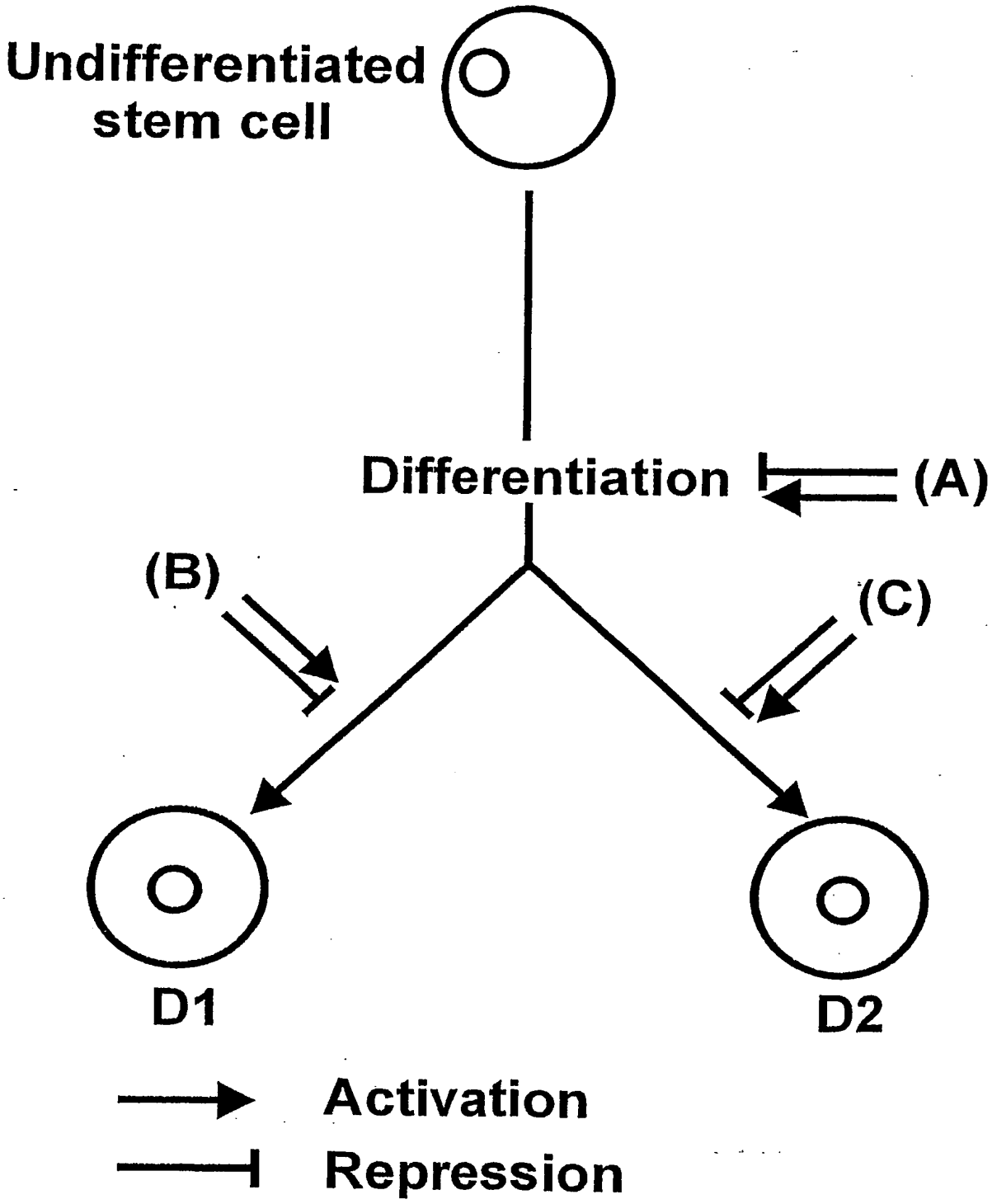
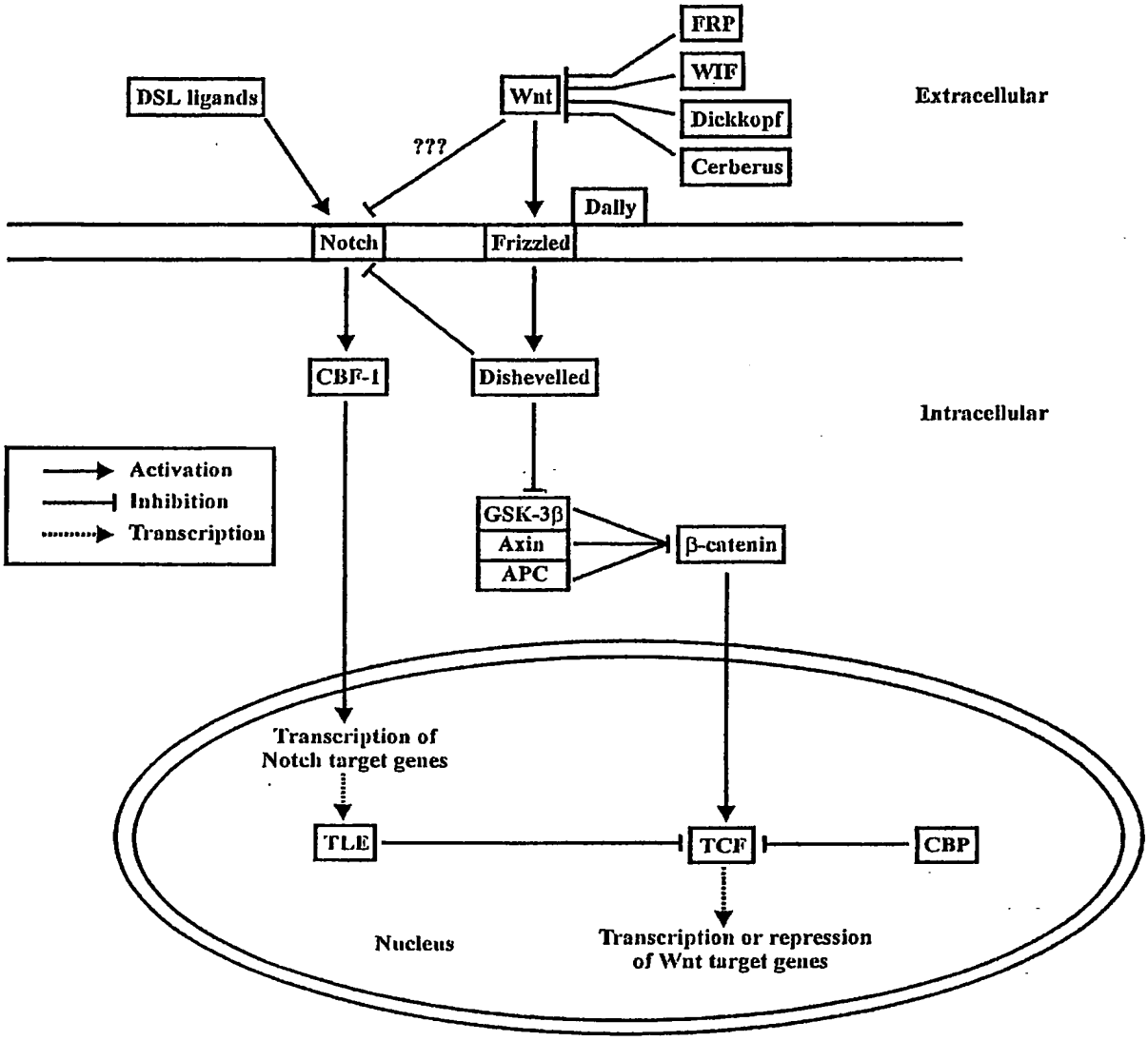


Fig 2



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD TO MODIFY DIFFERENTIATION OF PLURIPOTENTIAL STEM CELLS

(57) Abstract: We describe a method to manipulate the phenotype of stem cells, preferably pluripotential stem cells including nucleic acids and vectors used in said methods.

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INTERNATIONAL SEARCH REPORT

PCT/GB 03/00579

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/11 C07K14/47 C12N15/63 C12N15/85
C12N5/10 A61K31/713 A61K48/00 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, WPI Data, PAJ, SCISEARCH, CHEM ABS Data, BIOTECHNOLOGY ABS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ISO TATSUYA ET AL: "HERP, a novel heterodimer partner of HES/E(spl) in notch signaling." MOLECULAR AND CELLULAR BIOLOGY, vol. 21, no. 17, September 2001 (2001-09), pages 6080-6089, XP002253029 ISSN: 0270-7306	12,13
Y	abstract	1-11, 14-21

X	DATABASE EM_PAT [Online] EMBL; 29 October 2001 (2001-10-29), HILLMAN ET AL.: "transcription factors" XP002253031 retrieved from AX274948 accession no. EBI Database accession no. AX274948 abstract	12

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 September 2003	09. 01. 2004
Name and mailing address of the ISA	**Authorized officer**
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

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INTERNATIONAL SEARCH REPORT

PCT/GB 03/00579

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NAGEL ANJA C ET AL: "Neural hyperplasia induced by RNA interference with m4/malpha gene activity." MECHANISMS OF DEVELOPMENT, vol. 98, no. 1-2, November 2000 (2000-11), pages 19-28, XP002253030 ISSN: 0925-4773 page 19 page 23, column 1	1-11, 14-21
A	ELBASHIR S M ET AL: "Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 20, no. 23, 3 December 2001 (2001-12-03), pages 6877-6888, XP002225998 ISSN: 0261-4189 the whole document	1,8,11
A	PADDISON P J CAUDY A A HANNON G J: "Stable suppression of gene expression by RNAi in mammalian cells" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 99, no. 3, 5 February 2002 (2002-02-05), pages 1443-1448, XP002958887 ISSN: 0027-8424 abstract	1-11
A	UEDA R: "RNAI: A NEW TECHNOLOGY IN THE POST-GENOMIC SEQUENCING ERA" JOURNAL OF NEUROGENETICS, ELSEVIER, AMSTERDAM, NL, vol. 15, no. 3/4, 2001, pages 193-204, XP001147227 ISSN: 0167-7063 the whole document	1-21
T	DATABASE GSN [Online] DERWENT; 26 February 2003 (2003-02-26), TANG ET AL.: "Basic helix loop helix protein encoding sequence" XP002253032 retrieved from ABQ61092 accession no. EBI Database accession no. ABQ61092 abstract	12

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

PCT/GB 03/00579

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14 and 21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the RNAi molecules resp. cells or organs.

2. [X] Claims Nos.: 1-11, 14-21, all partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-21 all partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-11, 14-21, all partially

Present claims 1-11, 14-21 relate to a nucleic acid (and its use) defined by reference to a desirable characteristic or property, namely having inhibitory activity against the gene from which it has been derived. The claims cover all nucleic acids having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such nucleic acids. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the nucleic acid by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the inhibitory nucleic acids defined by the preparation process described on page 28, i.e. double stranded RNA molecules derived from a mRNA sequence defined by seq.id. 7-23, having a size of around 500 base pairs.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-21, all partially

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 7 or 8 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 7 or 8 and encodes a Notch signalling target gene (i.e. HERP) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 7 or 8 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 7 or 8 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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2. claims: 1-21, all partially

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 9 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 9 and encodes a Notch signalling target gene (i.e. HRY) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 9 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 9 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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3. claims: 1-21, all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 10, 11, 22, 23 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 10, 11, 22, 23 and encodes a Notch signalling target gene (i.e. HES) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 10, 11, 22, 23 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 10, 11, 22, 23 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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4. claims: 1-21, all partially

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 12 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 12 and encodes a Notch signalling target gene (i.e. HESR1) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 12 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 12 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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5. claims: 1-21, all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 13 or 14 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 13 or 14 and encodes a Notch signalling target gene (i.e. HEY) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 13 or 14 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 13 or 14 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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6. claims: 1-21, all partially

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 15 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 15 and encodes a Notch signalling target gene (i.e. HEYL) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 15 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 15 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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7. claims: 1-21, all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 16 or 17 or 18 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 16 or 17 or 18 and encodes a Notch signalling target gene (i.e. HRT) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 16 or 17 or 18 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 16 or 17 or 18 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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8. claims: 1-21, all partially

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 19 or 20 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 19 or 20 and encodes a Notch signalling target gene (i.e. CHF) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 19 or 20 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 19 or 20 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.

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9. claims: 1-21, all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An RNAi molecule derived from a nucleic acid comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 21 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 21 and encodes a Notch signalling target gene (i.e. GRIDLOCK) or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); a nucleic acid molecule encoding (part of) a gene which modulates stem cell differentiation comprising (i) a nucleic acid sequence as represented by SEQ.ID.No. 21 or (ii) a nucleic acid sequence which hybridizes to a nucleic acid sequence as represented by SEQ.ID.No. 21 and encodes a Notch signalling target gene or (iii) a nucleic acid sequence which is degenerate to (i) and (ii); an expression vector comprising said nucleic acid; use of said RNAi, said nucleic acid molecule or said vector for modulation of stem cell differentiation in vivo or in vitro and cells and organs obtained by said use.
